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# Research on the function and mechanism of survivin in heart failure mice model

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**Abstract. – OBJECTIVE: To observe the role of survivin in the heart failure mice model and to study its mechanism.**

**MATERIALS AND METHODS: 20 male C57BL/6J mice were selected and randomly divided into two groups: normal group (n=10) and heart failure group (n=10). After continuous modeling, the cardiac functions of mice in the two groups were detected via ultrasonic cardiogram equipment. The pathological conditions of hearts in the two groups were detected via hematoxylin and eosin (HE) staining. The expressions of survivin in heart tissues in both groups were detected via immunofluorescence method. The mRNA expressions of tumor necrosis factor-α (TNF-α), factor associated suicide (Fas) and factor associated suicide ligand (FasL) were detected via reverse transcription polymerase chain reaction (RT-PCR). The expressions of survivin in heart tissues of two groups were detected via Western blotting.**

**RESULTS: Ultrasonic cardiogram showed that the myocardial motion amplitude of mice in heart failure group was significantly decreased compared with that in normal group. HE staining further revealed the pathological conditions in heart failure group. RT-PCR showed that the mRNA expressions of TNF-α, Fas and FasL in heart failure group were significantly higher than those in normal group. Immunofluorescence and Western blotting showed that the expression of survivin in heart failure group was significantly higher than that in normal group.**

**CONCLUSIONS: Survivin expression is closely related to the occurrence and development of heart failure, indicating that survivin has an important value in the research on heart failure and the mechanism is related to inflammation and apoptosis.**

Key Words
Survivin, Heart failure, Inflammation, Apoptosis.

## Introduction

Survivin is a member of anti-apoptosis protein family in cells, which has the anti-apoptosis effect[1,2]. Heart failure refers to the cardiac systolic and diastolic dysfunction caused by different reasons, so the blood pumped out of the heart cannot meet the requirement of tissues when the circulating blood volume and vasomotor function are normal, or can only meet the requirement of metabolism when the ventricular filling pressure increases[3-5]. Heart failure is a kind of clinical syndrome with many characteristics, seriously affecting the human life. Therefore, the study on the pathogenesis and mechanism of heart failure has been the focus of cardiovascular disease[6,7]. So far, the pathogenesis of heart failure has not been clarified yet. In recent years, survivin, an anti-apoptosis protein, has been found to be closely related to the formation and development of heart failure[8]. In this experimental study, ultrasonic cardiogram and hematoxylin and eosin (HE) staining were used to detect the cardiac function and pathology of normal heart tissues and heart failure tissues. The mRNA and protein expressions of survivin in normal tissues and heart failure tissues, and also the relationship with inflammatory factors and apoptosis factors, were detected via reverse transcription polymerase chain reaction (RT-PCR), immunofluorescent staining and Western blotting, so as to investigate the expression differences of survivin in normal tissues and heart failure tissues and discuss roles and mechanisms of survivin in normal tissues and heart failure tissues, providing new ideas and directions for the genetic diagnosis and treatment of heart failure.

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## Materials and Methods

### Establishment of Heart Failure Mice Model

A total of 20 male C57BL/6J mice aged 4 weeks weighing 20±2 g was selected and randomly divided into normal group (n=10) and heart failure group (n=10). This study was approved by the Animal Ethics Committee of Sichuan University Animal Center. Mice in adriamycin group were treated with intraperitoneal injection of doxorubicin hydrochloride for heart failure modeling, and the cumulative dose of doxorubicin was 22 mg/kg. Mice in normal group were treated with intraperitoneal injection of the same volume of normal saline. Mice in the two groups were fed normally and then ultrasonic cardiogram was used to detect the cardiac functions of mice, followed by data analysis.

### Main Reagents

Doxorubicin hydrochloride (Pfizer, New York, NY, USA); bicinchoninic acid (BCA) protein quantification kit (Beyotime, Shanghai, China); Trizol total RNA extraction kit (Tiangen, Beijing, China); RT-PCR reverse transcription kit (Tiangen, Beijing, China); anti-β-Actin, anti-survivin monoclonal antibodies and immunofluorescence secondary antibodies (CST, Boston, MA, USA).

### Ultrasonic Cardiogram

Under the half-dose anesthesia, the spontaneous breathing of mice was maintained. The two-dimensional images of left ventricle were collected using the ultrasonic cardiogram equipment on the short-axis section of parasternal papillary muscles. The two-dimension-guided M-mode ultrasonic cardiogram of 10 cardiac cycles was obtained.

### Histopathological and Immunofluorescent Staining

The heart tissues of normal group and heart failure group were fixed with 10% formaldehyde for 48 h, embedded in paraffin and then prepared into 5 μm-thick sections. One part was used for HE histopathological staining, while the other part was used for survivin immunofluorescent staining. Paraffin sections were dewaxed using xylene and dehydrated using alcohol in gradient concentration, followed by antigen retrieval. Sections were washed with 0.01 M phosphate buffered saline (PBS) (pH7.4) for 3 times (5 min/time). Then, the sections were sealed in 10% bovine serum albumin (BSA) wet box for 30 min at 37°C. The appropriately-diluted fluorescence-labeled antibodies (1:70) were dropped onto the sections, which were placed in the wet box for incubation overnight at 4°C. After sections were washed with phosphate buffered saline (PBS) (pH7.4) for 3 times (5 min/time), the fluorescence secondary antibodies (diluted at 1:100) were dropped in a dark place for incubation in the wet box at 37°C for 2 h. Finally, sections were sealed via buffered glycerol, followed by observation and photography under upright fluorescence microscope.

### Real-Time PCR Analysis

An appropriate number of heart tissues in normal group and heart failure group were immediately transferred into 1 mL Trizol reagent for full tissue grinding to prepare them into homogenate. The homogenate was placed at room temperature for 5 min and centrifuged at 12000 g at 4°C for 5 min; then, the supernatant was carefully removed. Chloroform was added to the supernatant, the solution was mixed uniformly, placed at room temperature for 5 min and centrifuged at 12000 g at 4°C for 15 min; next, the supernatant was removed. The same volume of isopropanol was then added to the supernatant; the solution was placed at room temperature for 10 min, and centrifuged at 12000 g at 4°C for 10 min. The precipitate was taken out and added to 75% ethanol used to wash the RNA precipitate. Finally, RNase-free Water was added to dissolve the precipitate completely. Next, the $OD_{260}/OD_{280}$ ratio was determined and the RNA concentration was detected. Finally, the stepwise amplification was performed based on the primer sequence template shown in Table I according to the instructions, and the reaction products received the RT-PCR analysis.

### Western Blotting

The heart tissues collected in normal group and heart failure group were washed with ice normal saline. According to the instructions of

**Table I.** RT-PCR primer sequence of Survivin and β-actin mRNA.

| Gene name | Primer sequence |
|---|---|
| Survivin | 5′-3′ CCACTACGCATCGCATTCCA |
| | 3′-5′ CTTTCTCAAGGACCACCGCATCTC |
| β-actin | 5′-3′ GAGCCGGGAAATCGTGCGT |
| | 3′-5′ GGAAGGAAGGCTGGAAGATG |

the whole protein extraction kit, immunoprecipitation (IP) lysis buffer [containing phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors] was added for full tissue grinding to prepare it into tissue homogenate. Then, the tissue homogenate was centrifuged at 12000 g at 4°C for 10 min, and the supernatant was removed. The supernatant was centrifuged at 12000 g at 4°C for 20 min, and the supernatant was removed again. Protein quantification was performed according to the instructions of protein kit, and the protein sample containing the same amount of total protein was added to each well for loading, followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under the constant voltage of 220 V until the bromophenol blue reached the bottom of gel. According to the molecular weight of target protein, the gel was cut and the isolated protein was transferred onto the polyvinylidene fluoride (PVDF) membrane. The protein-coated PVDF membrane was sealed by 5% skim milk powder at room temperature for 3 h on the shaking table. Then, the corresponding primary antibodies (1:1000) were added for incubation at 4°C overnight. On the next day, after the membrane was washed with tween tris buffered saline (TTBS) fully (10 min/time, 3 times), the secondary antibodies (1:2000) were added for incubation at room temperature for 1 h. After the membrane was washed with TTBS (10 min/time, 3 times), electrochemiluminescence (ECL) developing solution was dropped for color development and photography.

### Statistical Analysis

The experimental data were presented as mean ± standard deviation (mean ± SD). The experimental results were analyzed using Statistical Product and Service Solutions (SPSS) 17.0 statistical software (Version X; IBM, Armonk, NY, USA). *t*-test was used for the comparison of means between the two groups, while one-way analysis of variance (ANOVA) was used for the comparison between groups followed by Post-Hoc Test (Least Significant Difference). *p*-test was used for pairwise comparison; $p<0.05$ suggested that the difference was statistically significant.

## Results

### Clinical and Ultrasound Results of Mice

At the end of the 10th week of experiment, mice in normal group had good grown, but 2 mice in heart failure group died, and the weights of 8 mice survived were significantly decreased compared with those in normal group ($p<0.01$), and the difference was statistically significant. The heart weight/body weight (HW/BW) ratios of mice in the two groups had no significant changes (Table II). The cardiac function indexes of mice were detected via the ultrasonic cardiogram and it showed that after the many-time intraperitoneal injection of small-dose doxorubicin, the myocardial motion amplitude of mice in heart failure group was significantly decreased compared with that in normal group.

**Table II.** Clinical and ultrasound results of mice.

| Item | Normal group | Heart failure group |
|---|---|---|
| SHW/BW | 0.48±0.12 | 0.46±0.04 |
| LVDd (mm) | 2.88±0.42 | 3.18±0.53 |
| LVDs (mm) | 1.88±0.26 | 2.38±0.46 |
| IVSd (mm) | 0.68±0.06 | 0.68±0.06 |
| LVPWd (mm) | 0.77±0.06 | 0.81±0.16 |
| LVEDV (ml) | 40.91±16.11 | 49.66±18.92 |
| LVESV (ml) | 11.41±4.45 | 20.11±8.99 |
| LVEF (%) | 72.11±3.88 | 59.52±5.13 |
| FS (%) | 34.17±2.88 | 26.17±3.32 |
| LV mass (mg) | 61.33±14.21 | 81.66±35.93 |
| LV mass/BW | 2.24±0.55 | 3.52±1.62 |

### Observation of Pathological Situations Via H&E Staining

HE stained sections of heart tissues in normal group and heart failure group were used to study the pathological differences of samples. Compared with the normal tissue sections, the volume of myocardial cells was increased, myocardial fiber proliferation occurred and the sizes of myocardial nuclei were different in sections of heart failure group (Figure 1). Some myocardial nuclei were large and stained deeply and there were many vacuoles near the nuclei in heart failure group.

### Detection of Survivin Expression Via Immunofluorescence Method

Survivin was rarely expressed in normal tissues, but highly expressed in heart failure tissues (Figure 2). This result revealed that survivin plays an important role in the occurrence and development of heart failure.

### RT-PCR Results

Total RNA was extracted from the normal tissue and heart failure tissue samples. RT-PCR

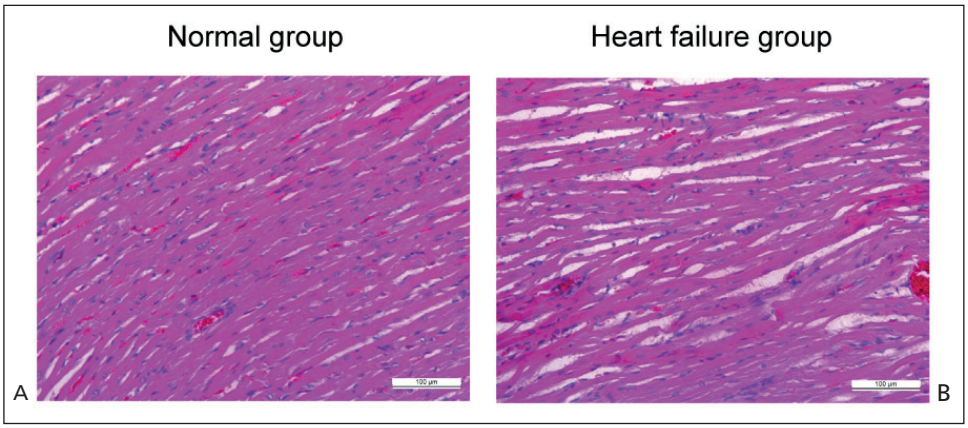


**Figure 1.** HE staining results of heart tissues in normal group (**A**) and heart failure group (**B**) (×200).

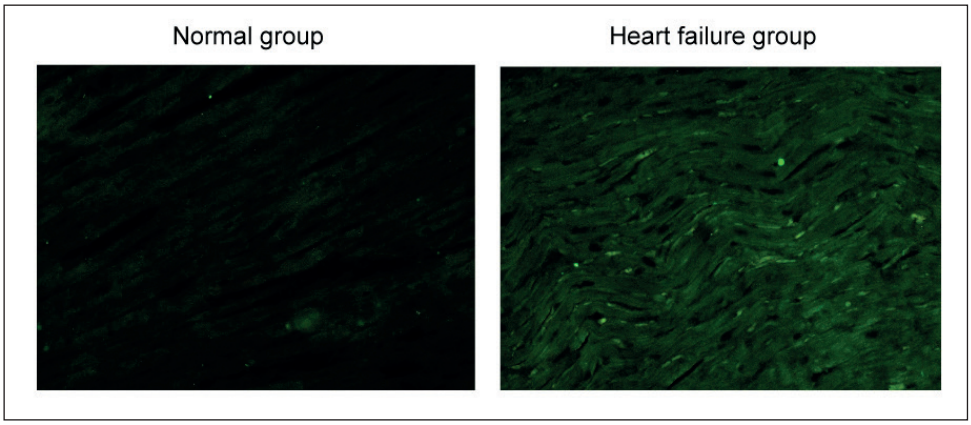


**Figure 2.** Expressions of survivin in heart tissues in normal group and heart failure group (×200).

showed that the expressions of necrosis factor-α (TNF-α), factor associated suicide (Fas) and factor associated suicide ligand (FasL) in heart failure tissues, were significantly higher than those in normal tissues (Figure 3). The overexpression of inflammatory factors and apoptosis factors in the pathogenetic process of heart failure lead to severe inflammation and apoptosis.

### *Protein Expressions of Survivin in Normal Tissues and Heart Failure Tissues*

Western blotting showed the protein expressions of survivin in normal tissues and heart failure tissues. The protein expression of survivin in heart tissues of heart failure group was significantly higher than that in normal tissue (Figure 4).

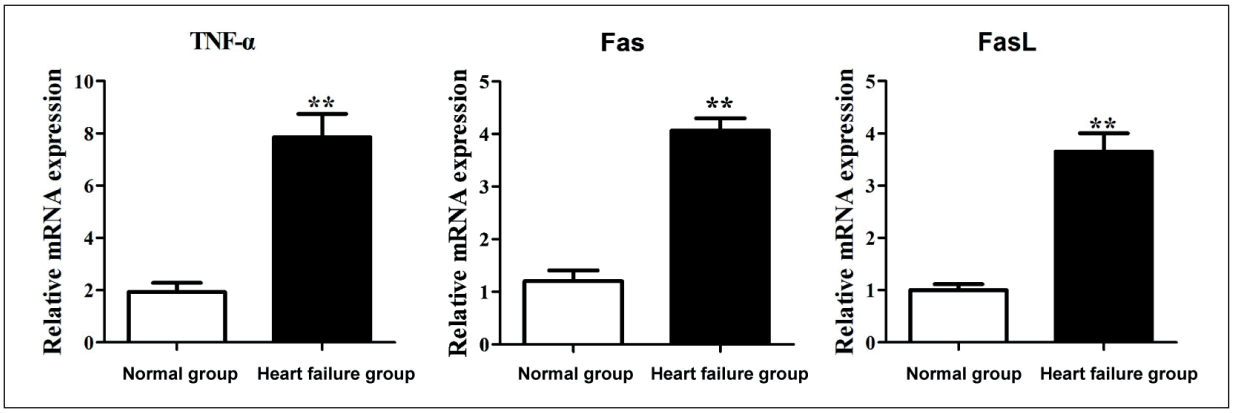


**Figure 3.** Gene expressions of TNF-α, Fas and FasL in normal tissues and heart failure tissues. Compared with normal group, * $p<0.05$, ** $p<0.01$ (n=3).

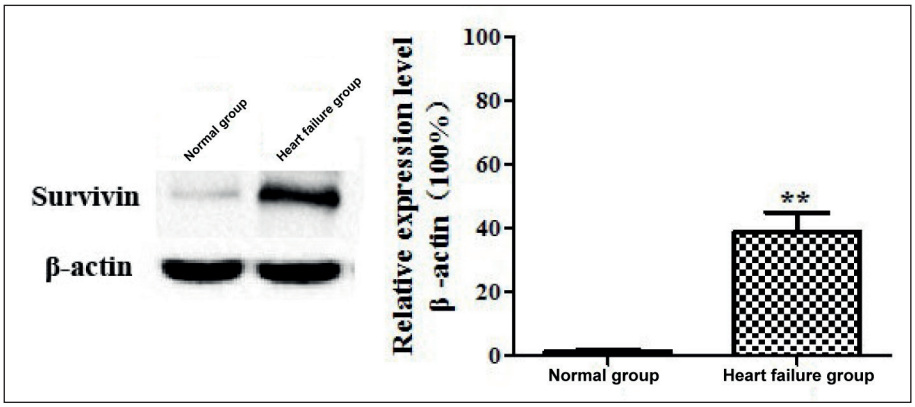


**Figure 4.** Protein expressions of survivin in normal tissues and heart failure tissues. Compared with normal group, *$p$<0.05, ** $p$<0.01 (n=3).

## Discussion

Heart failure is a kind of very complex clinical symptoms caused by ventricular filling or ejection impairment due to the cardiac structural or functional diseases[9]. Heart failure is the most common outcome of the development of acute or chronic myocardial injury[10]. For a long time, the heart failure has deeply been studied. So far, people have had a deep understanding of the pathogenesis, pathological and physiological mechanisms and clinical prevention and treatment of heart failure[11,12]. Survivin is a member of anti-apoptosis protein family with a variety of important biological functions and specific tissue expression profiles, which has rapidly attracted attention in the research field in recent years[13,14]. As a new member of anti-apoptosis protein family[15], survivin has become a new tumor molecular marker for clinical diagnosis of tumors so far[16-18]. Studies have found that survivin moves from the cytoplasm to the nucleus in the embryonic phase, which can initiate cells into the cell cycle, thereby inhibiting the apoptosis[19,20]. So survivin is conducive to the normal development of histiocytes. In this study, 20 male C57BL/6J mice were selected and randomly divided into two groups: normal group and heart failure group. Ultrasonic cardiogram equipment was used to detect the cardiac functions of mice in the two groups. Ultrasonic cardiogram showed that the myocardial motion amplitude of mice in heart failure group was significantly decreased compared with that in normal group. HE histopathological staining showed the pathological changes in heart failure tissues very intuitively. The mRNA expression of TNF-α (a kind of common inflammatory factor), Fas and FasL (apoptotic bodies), were detected *via* RT-PCR. The experimental results showed the inflammation and aggregation of apoptotic bodies in heart failure group. Immunofluorescence and Western blotting showed that the expression of survivin in heart failure tissues was high, which was significantly higher than that in normal tissues.

## Conclusions

The abnormal expression of survivin expression is closely related to the occurrence and development of heart failure, indicating that survivin has an important value in the research on heart failure and the mechanism is related to inflammation and apoptosis. This study deepened the understanding of the molecular mechanism of survivin in heart failure. Survivin, as a target molecule, can provide a new direction and scientific guidance for the treatment of heart failure, hematopoiesis regulation and organ transplantation.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

## References

1) Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3: 917-921.
2) Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003; 22: 8581-8589.
3) Soylu MO, Altun I, Basaran O, Uzun Y, Dogan V, Ergun G, Akin F, Biteker M. Impact of QRS morphology on heart rate turbulence and heart rate variability after cardiac resynchronization therapy in patients with heart failure. Eur Rev Med Pharmacol Sci 2016; 20: 317-322.

4) Li Z, Bing OH, Long X, Robinson KG, Lakatta EG. Increased cardiomyocyte apoptosis during the transition to heart failure in the spontaneously hypertensive rat. Am J Physiol 1997; 272: H2313-H2319.
5) Gong HB, Wang L, Lv Q, Wang J. Improved systolic function of rat cardiocytes during heart failure by overexpression of SERCA2a. Eur Rev Med Pharmacol Sci 2016; 20: 1590-1596.
6) Abbate A, Biondi-Zoccai GG, Baldi A. Pathophysiologic role of myocardial apoptosis in post-infarction left ventricular remodeling. J Cell Physiol 2002; 193: 145-153.
7) Gonzalez A, Fortuno MA, Querejeta R, Ravassa S, Lopez B, Lopez N, Diez J. Cardiomyocyte apoptosis in hypertensive cardiomyopathy. Cardiovasc Res 2003; 59: 549-562.
8) Zhou M, Gu L, Li F, Zhu Y, Woods WG, Findley HW. DNA damage induces a novel p53-survivin signaling pathway regulating cell cycle and apoptosis in acute lymphoblastic leukemia cells. J Pharmacol Exp Ther 2002; 303: 124-131.
9) Noton EA, Colnaghi R, Tate S, Starck C, Carvalho A, Ko FP, Wheatley SP. Molecular analysis of survivin isoforms: evidence that alternatively spliced variants do not play a role in mitosis. J Biol Chem 2006; 281: 1286-1295.
10) Rodriguez M, Lucchesi BR, Schaper J. Apoptosis in myocardial infarction. Ann Med 2002; 34: 470-479.
11) Santini D, Abbate A, Scarpa S, Vasaturo F, Biondi-Zoccai GG, Bussani R, De Giorgio F, Bassan F, Camilot D, Di Marino MP, Feroce F, Baldi F, Silvestri F, Crea F, Baldi A. Surviving acute myocardial infarction: survivin expression in viable cardiomyocytes after infarction. J Clin Pathol 2004; 57: 1321-1324.
12) Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. Circulation 1995; 91: 161-170.
13) Kami K, Doi R, Koizumi M, Toyoda E, Mori T, Ito D, Fujimoto K, Wada M, Miyatake S, Imamura M. Survivin expression is a prognostic marker in pancreatic cancer patients. Surgery 2004; 136: 443-448.
14) Ku JH, Kwak C, Lee HS, Park HK, Lee E, Lee SE. Expression of survivin, a novel inhibitor of apoptosis, in superficial transitional cell carcinoma of the bladder. J Urol 2004; 171: 631-635.
15) Bolton MA, Lan W, Powers SE, McCleland ML, Kuang J, Stukenberg PT. Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation. Mol Biol Cell 2002; 13: 3064-3077.
16) Wheatley SP, Carvalho A, Vagnarelli P, Earnshaw WC. INCENP is required for proper targeting of Survivin to the centromeres and the anaphase spindle during mitosis. Curr Biol 2001; 11: 886-890.
17) Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998; 396: 580-584.
18) Vaira V, Lee CW, Goel HL, Bosari S, Languino LR, Altieri DC. Regulation of survivin expression by IGF-1/mTOR signaling. Oncogene 2007; 26: 2678-2684.
19) Sommer KW, Schamberger CJ, Schmidt GE, Sasgary S, Cerni C. Inhibitor of apoptosis protein (IAP) survivin is upregulated by oncogenic c-H-Ras. Oncogene 2003; 22: 4266-4280.
20) Kawasaki H, Altieri DC, Lu CD, Toyoda M, Tenjo T, Tanigawa N. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res 1998; 58: 5071-5074.