Original Article

Reduction of c-kit positive cardiac stem cells in patients with atrial fibrillation

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Abstract

Background

We aimed to determine expression patterns of cardiac stem cells in the left atrium (LA) tissue from patients with atrial fibrillation.

Methods

LA appendages were obtained during open-heart surgery and processed for explant cell culture and tissue analysis (n = 319). The total number of grown cells and c-kit positive cells were analyzed by flow cytometry after 4 weeks of culture. The remaining tissue was used for Masson's trichrome staining to determine the area of the fibrosis.

Results

The diameter of the LA, as measured by echocardiography, was significantly larger in the AF group than in the SR group. RT-PCR analysis revealed higher expression of collagen in the AF group and an increase in the expression of basic fibrosis growth factor and transforming growth factor-2 and -3. Masson's trichrome staining showed progression of fibrosis in the AF tissue. In addition, the expression of apoptosis related genes were significantly higher in AF group. There was no difference in the expression of connexin-40 between groups, while the expression of connexin-43 was decreased and

that of connexin-45 was increased in the AF group. The total numbers of grown cells as well as c-kit positive cells after 4 weeks of cardiac tissue culture were significantly lower in the AF group.

Conclusion

Progression of remodeling in LA tissue was observed in AF patients. The number of c-kit positive cells cultured from LA appendages was reduced in AF patients, suggesting impairments in self-renewal.

Introduction

Atrial fibrillation (AF) is the most common arrhythmia that occurs due to multiple factors such as aging, gender, obesity, hypertension, or heart disease.[1-5] Prolonged AF causes fibrotic changes or dilatation of the left atrium (LA).[6,7] known as remodeling. Causation of AF is associated with mechanical stretching of the left atrium (LA),[8] changes in ion channel expression,[9] or autonomic nerve activity.[10] In addition, connexin family, main components of gap junction proteins, play a central role in the electrical remodeling associated with AF, and an alteration in the expression or distribution of connexin(s) has been observed in the heart of patients with AF.[11-13] These structural or electrical remodeling processes enhance intrinsic consequences of arrhythmia, resulting in persistent AF. Many studies suggest that inflammation is responsible for the development of remodeling. In addition, recent study demonstrated that local expression of micro-RNA may contribute atrial remodeling in patients with chronic af,[14] however the underlying mechanism is still unclear.

The heart is no longer considered to be a terminally differentiated organ and it is now known that heart cells are renewed at a certain rate annually.[15,16] Recently, resident cardiac stem cells (CSCs) have been identified[17] and these are considered to be involved in cardiac regeneration or maintenance.[18,19] C-kit positivity is a general marker for CSC identification, and this protein plays a central role in self-renewal and the ability to differentiate into cardiac cells.[20] The expression of c-kit positive cells is associated with age, gender, and serum level of brain natriuretic peptide.[21,22] Importantly, c-kit expression is increased when the cardiac tissue is damaged, such as in the case of myocardial infarction or end-stage heart failure in animal or human hearts.[22-24] Therefore, if LA tissue in patients with AF is damaged, expression of c-kit positive cells may be affected. To test this, we investigated whether there were any changes in functional activity of c-kit positive cells in patients with AF, using human atrial tissue obtained during open-heart surgery.

Methods

Patients

A signed written consent was obtained from patients according to a protocol approved by the Internal Review Committee on Ethics of Human Investigation of the Juntendo University Hospital prior to the collection of tissue samples. Patients who underwent open-heart surgery between September 2011 and February 2014 were enrolled for this study. The patients were divided into two groups: persistent atrial fibrillation (AF) and sinus rhythm (SR). Patients diagnosed with paroxysmal AF, patients with pacemaker implants, patients on hemodialysis due to chronic renal failure, patients with history of previous open-heart surgery, and patients who had received chest irradiation for cancer therapy were excluded from this study.

Tissue collection and culture of cardiac outgrowth cells

The left atrium appendage was obtained during cardiac surgery. The samples were immersed in cardioplegic solution (Miotecter®, Mochida Pharma, Tokyo, Japan) immediately after tissue dissection and stored until further use. The sample was cut into small pieces and trimmed to a final weight of 20-30 mg for primary cell culture. Any remaining piece of sample was immersed in RNAlater solution (Life Technologies, Tokyo, Japan) for RNA isolation or 4% paraformaldehyde for histochemical analysis. The sample for culture was minced into small pieces (80-100 pieces) and digested for 5 minutes with 0.05% trypsin-EDTA (Sigma-Aldrich, Tokyo, Japan). After washing with phosphate-buffered saline (PBS, Wako, Tokyo, Japan) the explants were placed onto two fibronectin-coated dishes (BD Biosciences, Tokyo, Japan) in Iscove's modified Eagle's medium (Life Technologies) containing 10% fetal bovine serum (Thermo Scientific, Yokohama, Japan), 1% penicillin-streptomycin (Life Technologies), and 2 mM L-glutamine (Life Technologies). The media were changed every 3-4 days. A primary outgrowth of cells growing out radially in a monolayer from the cardiac tissue was seen after 4 weeks of culture. These cells were harvested with 0.05% trypsin-EDTA and counted. A portion of the cells was used for analysis by flow cytometry and the remaining cells were stored at −80°C.

Flow cytometry

A portion of the harvested outgrowing cells was fixed in 4% paraformaldehyde in PBS (Sigma) for 15 minutes. The cells were then washed twice with PBS, followed by blocking with 1% bovine serum albumin (Iwai Chemicals Company, Tokyo, Japan) for 30 minutes. Cells were incubated with phycoerythrin-conjugated anti-human c-kit antibody (BD Bioscience, Franklin Lakes, NJ, USA) for 2 hours at 4°C. Cells were then washed with 0.1% bovine serum albumin twice followed by one PBS wash. The cells were counted using a FACSCalibur flow cytometer and CellQuest software (BD Bioscience).

Reverse transcription polymerase chain reaction (RT-PCR)

mRNA was extracted from the heart tissue by using an RNA extraction kit (Qiagen, Tokyo Japan). Extracted RNA was then reverse transcribed into cDNA (iScript cDNA Synthesis Kit, Bio-Rad, Tokyo, Japan). PCR and subsequent analysis were carried out using a SYBR-green kit (Life Technologies) and the HT-7700 thermal cycler system (Life Technologies). Primers were designed to amplify and quantify GAPDH and c-kit. The relative abundance of the target gene was obtained by calculating gene-specific mRNA expression normalized to the internal control GAPDH.

Masson's trichrome staining

Masson's trichrome staining was performed to quantify the area of fibrotic change. After fixation in 4% paraformaldehyde, the tissue was dehydrated by immersing in 10%, 15%, and 20% sucrose solutions in succession prior to embedding in OCT compound (Sakura Finetek, Tokyo, Japan), and then stored at -80°C. Subsequently, each sample was cut into 5-μm sections using a cryostat. Sections were a mounted on a glass slide and the slides were then left immersed into Bouin solution (Muto Pure Chemicals, Tokyo, Japan) overnight at room temperature. The slides were processed the next day using a staining kit (Sigma-Aldrich) according to the manufacturer's instructions. Quantification analysis of the fibrotic area was performed using ImageJ software developed by NIH.

Statistical analyses

The Kolmogorov-Smirnov test was used to assess the normality of data distribution. Area analysis of Masson's staining was evaluated by the Welch's *t*-test using Statcel 3 (O.M.S. publisher, Saitama, Japan). The difference in the mRNA expression between groups was analyzed by the Mann-Whitney U-test using SPSS 22.0 (IBM-Japan, Tokyo, Japan). All data are represented as the mean ± standard deviation (SD). Statistical significance was set at a level of $p < 0.05$.

Results

Patient background

A total of 319 cardiac specimens obtained from patients including those with chronic AF ($n = 104$), were analyzed. The details of the clinical background of the patients are shown in Table 1. The AF group had older patients ($p < 0.01$), more female patients ($p < 0.01$), less triglyceride values ($p < 0.05$), higher serum creatinine values ($p < 0.05$), higher values of serum brain natriuretic peptide (BNP, $p < 0.01$), and higher preoperative NYHA classification ($p < 0.01$). Notably, overall there were more male patients in both the SR (71.0%) and the AF groups (53.3%). In addition, the SR group included a significantly higher number of patients who had undergone coronary artery bypass surgery (CABG) due to ischemic heart disease ($p < 0.01$), while the AF group showed a higher rate of mitral valve surgery ($p < 0.01$). Further, 89.5% of AF patients had a history of maze procedure.

Structural and electrical remodeling of the left atrium

The preoperative echocardiogram data is shown in Table 2. Patients with AF had significantly larger left atrial diameter (LAD). In addition, there was no significant difference in the left ventricular ejection fraction between groups (SR vs. AF: 59.0 ±

15.3% vs. 59.3 ± 12.1%, $p = 0.99$), or in the left ventricle diameter during contraction (left ventricular diameter in diastolic phase and systolic phase: LVDd/LVDs).

RT-PCR analysis of the tissue (Figure 1) revealed that the expression of type 1 collagen was significantly higher in AF group (2.2 -fold, $p < 0.01$) along with an increase in expression of beta-fibrosis growth factor (1.41 -fold, $p < 0.01$), and transforming growth factor-2 (2.5 -fold, $p < 0.01$) and 3 (3.3 -fold, $p < 0.01$) compared with the SR group (Figure 1A). In addition, the apoptosis related genes CASP3 (13.3-fold, $p < 0.01$) and CASP9 (3.7-fold, $p < 0.01$), were significantly high in the AF group (Figure 1B).

Representative images of Masson's trichrome staining of LA appendage and area analysis for fibrosis are shown in (Figure 2A-C). For fibrosis analysis: the area of fibrosis (blue) was divided by the area of the whole tissue (blue and red). The results show that the area of fibrosis was significantly larger in the AF heart compared with the SR group (SR vs. AF: 9.8 ± 3.4% vs. 13.8 ± 6.9%, $p < 0.05$, n =20 each).

We next tested if AF resulted in modulation of expression of gap junction proteins (Figure 3). RT-PCR data suggested that the expression of connexin-40 (Cx40) was lower in the AF group however this decrease was not statistically significant (0.9-fold vs. SR, $p = 0.05$). In addition, the expression of Cx43 was significantly less (0.8-fold, p

< 0.01) and that of Cx45 were significantly increased (1.5-fold, $p < 0.01$) in the AF group. These data demonstrate that more progressive structural and electrical remodeling of the LA occurred in AF compared to SR.

Cell culture from cardiac specimens

The samples of cardiac tissue were processed for explant culture (N = 254). The number of the cells was counted to determine the rate of proliferation after 28 days (Figure 4a). The number was less in the tissue obtained from AF patients compared to those from SR (SR vs. AF: 9.6 ± 4.3 vs. 8.6 ± 4.4 [per mg], $p < 0.01$) patients. FACS analysis of c-kit expression to quantify the stem cell population among the cultured cells showed that the estimated number of c-kit positive cells was also lower in samples from AF patients (SR vs. AF: 0.15 ± 0.12 vs. 0.13 ± 0.18 [per mg], $p < 0.01$, figure 4b), although the rate of c-kit positive cells contained in outgrowth cells was not different between groups (SR vs. AF: 1.22 ± 0.88% vs. 1.15 ± 0.58%, $p = 0.61$, figure 4c).

Sub-analysis in the patients with mitral valve disease

Since the patient with AF included significantly more patients with mitral valve disease (MVD) whom surgical treatment was required. To minimize the effect of mechanical stretch to ability of cell proliferation or to c-kit expression, we further performed sub-group analysis in those patients. The characteristic of the patients and pre-operative echo data were shown in Table 3. The mean age of AF group was still significantly higher, however gender bias and pre-operative dyslipidemia bias were removed in this comparison. In addition, the value of serum BNP was not different between groups, although NYHA classification was still higher in the AF group. The ratio of the patients who required CABG due to ischemic heart disease was now not significant between groups, while procedure for aortic valve or tricuspid valve was performed significantly more in the AF groups. Preoperative echocardiogram showed that significant enlargement of LAD was observed in the AF group, although both groups had MVD. Furthermore, the size of left ventricular was significantly higher in the AF group in this sub-analysis. When analysis of outgrowth cells, the total number of grown cells was less in the tissue from AF patients compared to those from SR patients (SR vs. AF: 9.8 ± 3.7 vs. 8.8 ± 4.2 [per mg], $p < 0.01$). FACS analysis of c-kit expression in outgrowth cells showed that the estimated number of c-kit positive cells was also lower in AF patients (SR vs. AF: 0.15 ± 0.15 vs. 0.13 ± 0.10 [per mg], $p < 0.01$, figure 5b), although the rate of c-kit positive cells contained in outgrowth cells was not different between groups (SR vs. AF: 1.18 ± 1.04% vs. 1.12 ± 0.62%, $p = 0.34$, figure 5c).

Discussion

We demonstrated that the expression of c-kit positive cells in LA tissue was decreased in patients with AF, indicating impairment in self-repair mechanisms of the heart. In this study, patients with AF were significantly older and more likely to be male, and AF patients had high serum creatinine values, which is consistent with previous reports.[1-4] Very few patients with AF had a previous history of ischemic heart disease, and as a result, limited patients had a history of undergoing procedures used solely for ischemic heart disease (CABG alone) (3 cases; 2.9%) compared with those in the SR (56 cases; 26.2%) group. However, the use of preoperative medication like statins did not produce statistical differences, although dyslipidemia and higher triglyceride values were more common in the SR group.

Alteration in gap junctional proteins in AF

Persistent AF is known to result in electrical and structural remodeling of the heart.[6,7] Cx40 and Cx43 are the major connexins in the human heart.[26] The expression of Cx40 is known to be reduced in AF.[7,25] Other groups have demonstrated that genetic polymorphisms are associated with the causation of AF.[26,27] However, phosphorylation changes in Cx40 or 43 have been observed in patients with AF[25] and the function of Cx43 is known to be dependent on its phosphorylation state, and stress, e.g. ischemia/hypoxia-induced dephosphorylation of the Cx43 protein,[25,28] and the ratio of phosphor/dephospho-Cx43 are correlated with cardiac function.[29] Notably, electrical stimulation of the isolated rat heart induces dephosphorylation of Cx43 without changing the total amount of Cx43 protein,[30] suggesting that phosphorylation of Cx43 reflects electrical remodeling of the heart. In addition, a previous study demonstrated that the amount of phosphorylated-Cx43 was correlated with left ventricular function in ischemic heart.[31] In this study, we compared the expression of three different types of gap junction proteins: Cx40, Cx43, and Cx45. A decrease in Cx43-expression, an increase in Cx45 expression, and no significant reduction in Cx40 expression were seen in AF hearts. Although, we did not assess the amount of phospho- or dephospho- Cx43 in this study, further investigation may demonstrate the relationship between phosphorylation status of connexin and c-kit expression.

Structural remodeling in the AF heart

The expression of type 1-collagen mRNA was high in the AF group, and this was confirmed by histological analysis using Masson's trichrome staining. Echocardiogram analysis also demonstrated significant dilatation of LA diameter in the AF group. Furthermore, serum levels of brain natriuretic peptide (BNP) were assessed. BNP is secreted mainly by ventricular cardiomyocytes, and in clinical settings, serum BNP is widely used as a marker for cardiac damage.[32-35] In our study, not only was the serum BNP high in the AF group, but the expression of BNP-mRNA was also significantly high in the atrial tissue of the AF group. Interestingly, the mRNA expression of atrium natriuretic peptide (ANP), which is secreted from the atrial tissue, was not significantly different between the groups. In addition, mRNA expression of caspase-3 (CASP3) and -9 (CASP9) were significant high in the AF group in our study. While the factors influencing the life span of cardiomyocytes have not yet been defined, overcontraction or stress could be important factors, since the energy for cardiomyocyte activity is provided mainly by the mitochondria. Although heart rates were not significantly different between groups, cardiomyocytes in the LA contract more than 300-600 times per minute during AF, which would require high energy consumption.

Attenuation of cardiac stem cell proliferation

The heart contains cardiac stem cells (CSCs), which contribute to self-renewal. An increased of the expression of CSCs is observed when the heart is damaged.[22-24,36]

However, our data demonstrated that the ratio of c-kit positive cells contained in outgrowth cells did not increase when AF was existence. As a result, both of the total number of the grown cells and the number of c-kit positive cells after 28 days of explant culture were decreased in the AF group. This trend was observed consistently in sub-analysis on the patients with mitral valve disease. These results suggest that self-repairing capability was disturbed in AF tissue despite tissue damage. In addition, our analysis also points towards a limited capability for renewal, as the average percentage of c-kit positive cells among the cardiac outgrowth cells was 1.3% (0.6 - 8.6%). Therefore, insufficient supply of cells cannot meet high demands and results in remodeling of the tissue. In clinical settings, the heart never recovers by itself after injury such as myocardial infarction, likely because the turnover rate of cardiomyocytes is reported to be very low in aging people (0.04-0.3% annually).[15,16] Further, recent studies suggested that c-kit positive CSCs possess limited ability to regenerate cardiomyocytes.[37]

Potential mechanisms that inhibit AF-induced remodeling

Our results also demonstrated a significant increase in the expression of basic fibroblast growth factor (bFGF), transforming growth factor beta-2, and -3 (TGF-β2, β3) in AF atria. These factors are known to be upregulated when cardiac tissue is injured and they induce the expression of collagen in an attempt to repair the wounded tissue.[38,39] These results indicated that a healing mechanism is activated in the AF heart, resulting in collagen-associated remodeling. If the self-renewal mechanism mediated by CSCs is accelerated, this may prevent fibrotic repair of the heart, resulting in a reduction of remodeling. Future studies should focus on detecting the factor(s) that activate CSCs. This will enable the development of techniques to reduce cardiac remodeling.

Limitations

There were some limitations in this study. First, although the patients with paroxysmal AF were excluded from this study, the duration of the AF was varied in patients. Second, the left atrial tissue used in this study was obtained from left atrial appendages, which may have a different morphology compared with the tissue close to foci of arrhythmia. In the future, a comparison between the appendage and the body of the apex may help clarify any differences between the foci and remote areas, even in the same atrium.

Conclusion

We demonstrated that cell growth and total number of c-kit positive cells were reduced in cell cultures derived from LA tissues obtained from AF patients.

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Disclosure

The authors declare no conflicts of interest

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Figure legends

Figure 1. Gene expression of fibrosis related factors, natriuretic peptides and apoptosis related peptide

(A) RT-PCR analysis demonstrated that mRNA of type-1 collagen (coll-1) was significantly increased (in the left atrium appendage of patients with AF compared with those of the SR group (2.2 ± 1.6 -fold, $p < 0.01$). The expression of basic-fibroblast growth factor (bFGF), and transforming growth factor-beta2 and 3 (TGFβ2, TGFβ3) were also increased in AF (bFGF: 1.4 ± 0.5 -fold, TGFβ2: 2.5 ± 2.7 -fold, TGFβ3: 3.3 ± 2.1 -fold, $p < 0.01$ vs. SR, respectively), although the expression of TGFβ1 was no significantly difference. The mRNA expression of brain natriuretic peptide (BNP) was significantly increased in AF (2.8 ± 2.1 -fold, $p < 0.01$ vs. SR). In contrast, no difference was seen in the expression of atrium natriuretic peptide (ANP) (1.1 ± 0.2 -fold, $p = 0.21$, vs. SR).

(B) Expression of apoptosis related genes, CASP3 and CASP9, were significantly high in the AF group (CASP3: 13.3 ± 4.9-fold, CASP9: 3.7 ± 3.4-fold, $p < 0.01$ vs. SR, respectively).

Figure 2. Masson's trichrome staining analysis

(A)(B) Representative image of Masson's trichrome staining of a left atrium appendage obtained from a patient with SR and from a patient with AF. (C) Fibrotic area (blue) analysis. AF patient showed significant larger area of fibrosis than the SR patient (SR vs. AF: 9.8 ± 3.4 % vs. 13.8 ± 6.9 %, $p < 0.05$).

Figure 3. Comparison of the expression of mRNA of gap junction proteins

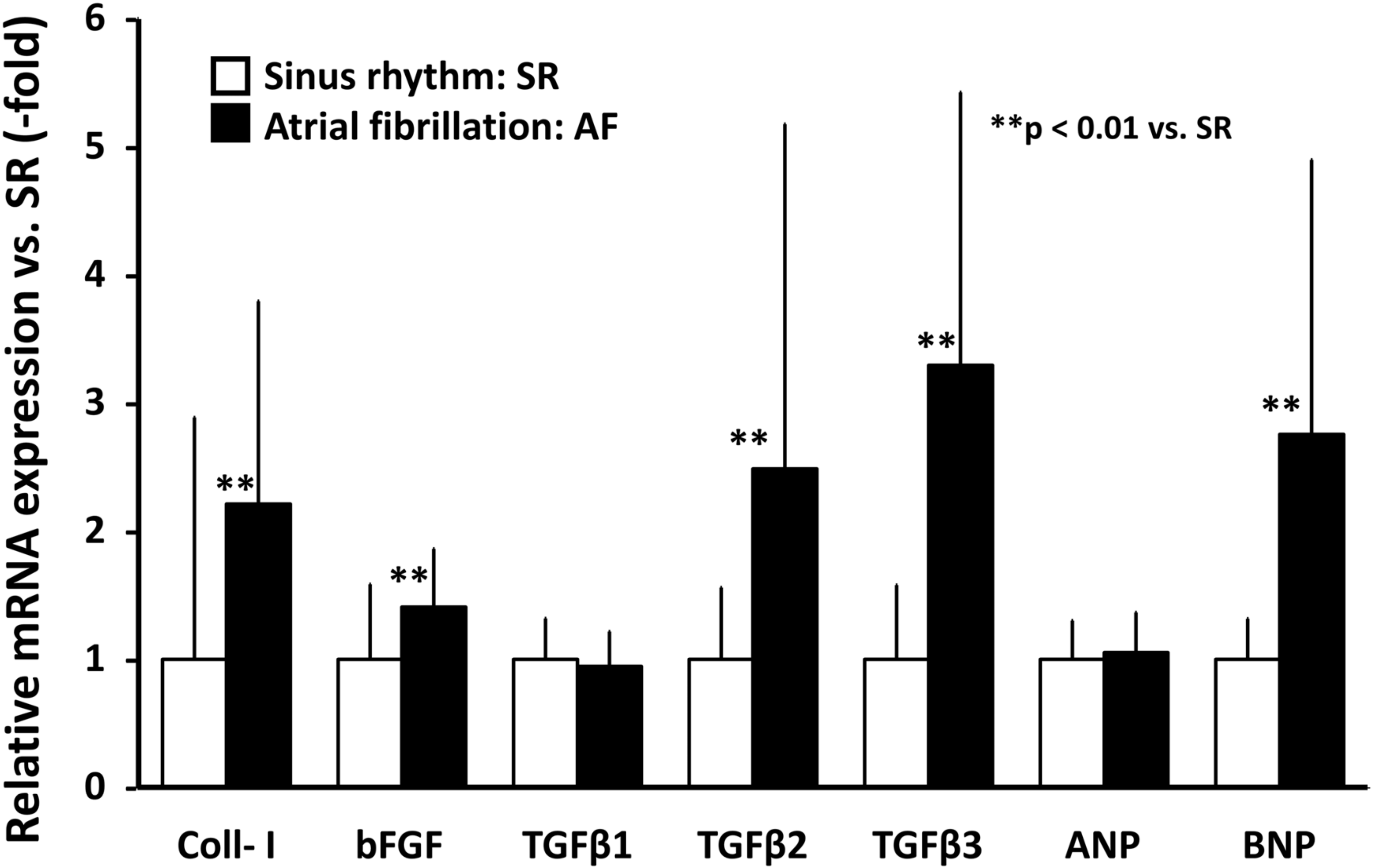
The mRNA expression of gap junction proteins in patients with AF compared to those with SR, although there was reduction in the expression of connexin 40 (Cx40) in AF patients, there was no significant difference between groups (AF: 0.9 ± 0.7 -fold, $p = 0.41$, vs. SR). The expression of Cx43 mRNA was significantly lower (0.8 ± 0.3 -fold, $p < 0.05$) and the expression of Cx45 mRNA was significantly higher (1.5 ± 0.7 -fold, $p < 0.01$) in the AF group.

Figure 4. Characteristics of the outgrowth cells cultured from cardiac explant

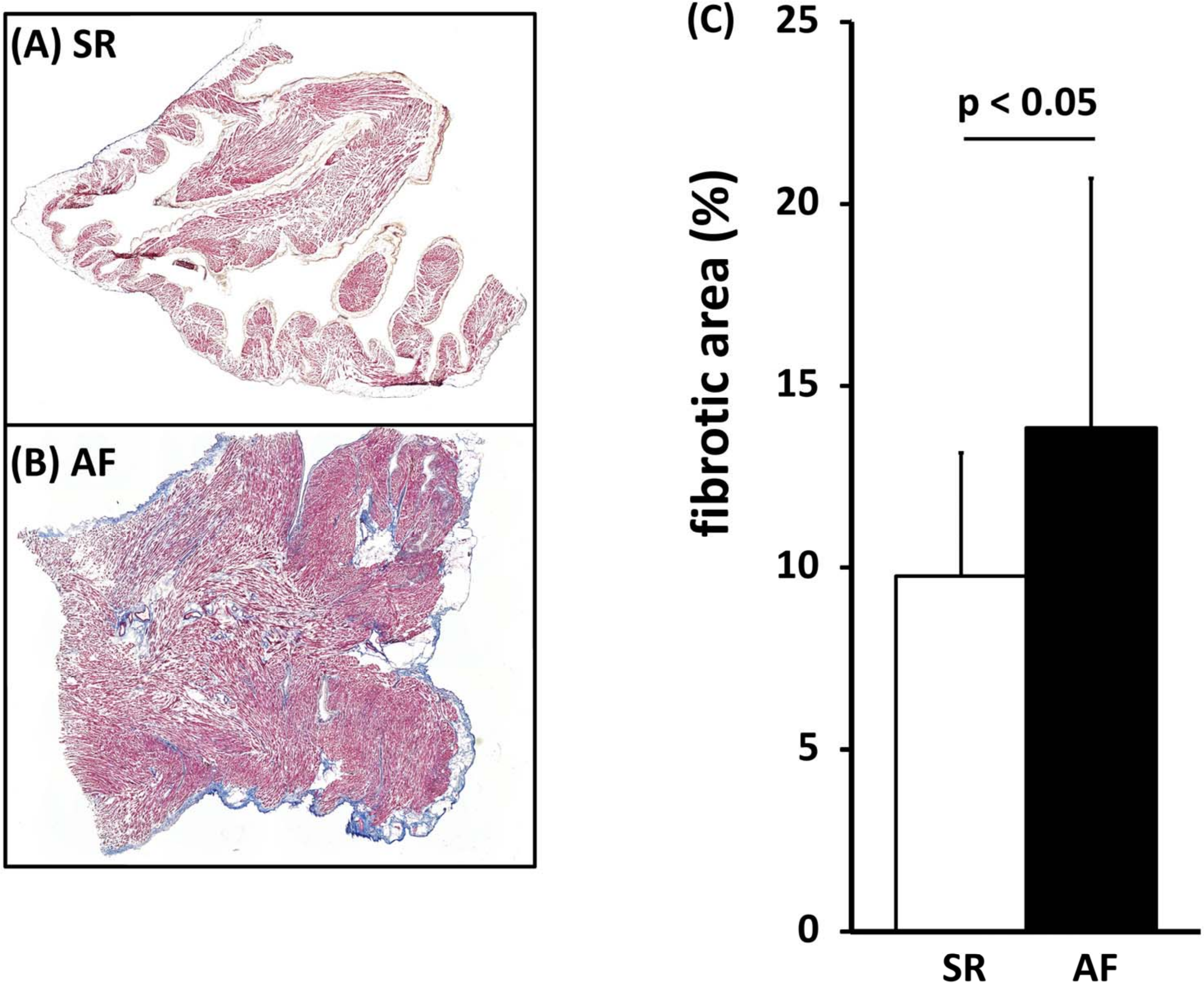
The character of the cells at 28 days after explant culture of the LA tissue was compared between the patients from SR and those with AF. (A) Total number of the cells cultured from 1 mg of cardiac specimen was significantly lower from AF compared with SR (SR vs. AF: 9.6 ± 4.3 vs. 8.6 ± 4.4 [per mg], $p < 0.01$). (B) The estimate number of c-kit positive cells (c-kit$^+$) was also significantly lower from AF (SR vs. AF: 0.15 ± 0.12 vs. 0.13 ± 0.10 [per mg], $p < 0.01$), while the ratio of c-kit$^+$ cells was not statistically different between groups (C).

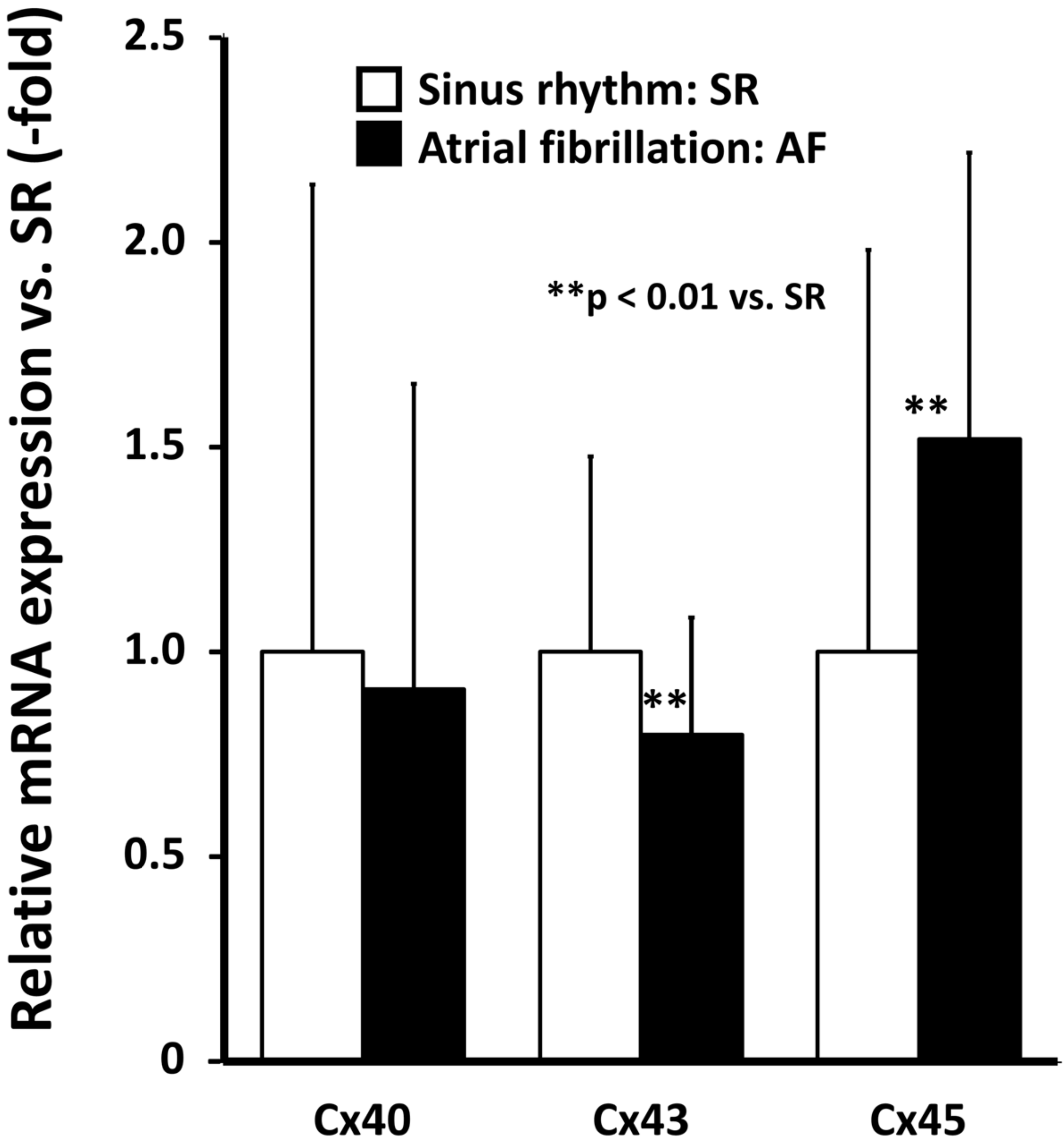
Figure 5. Characteristics of the outgrowth in the patients with mitral valve disease

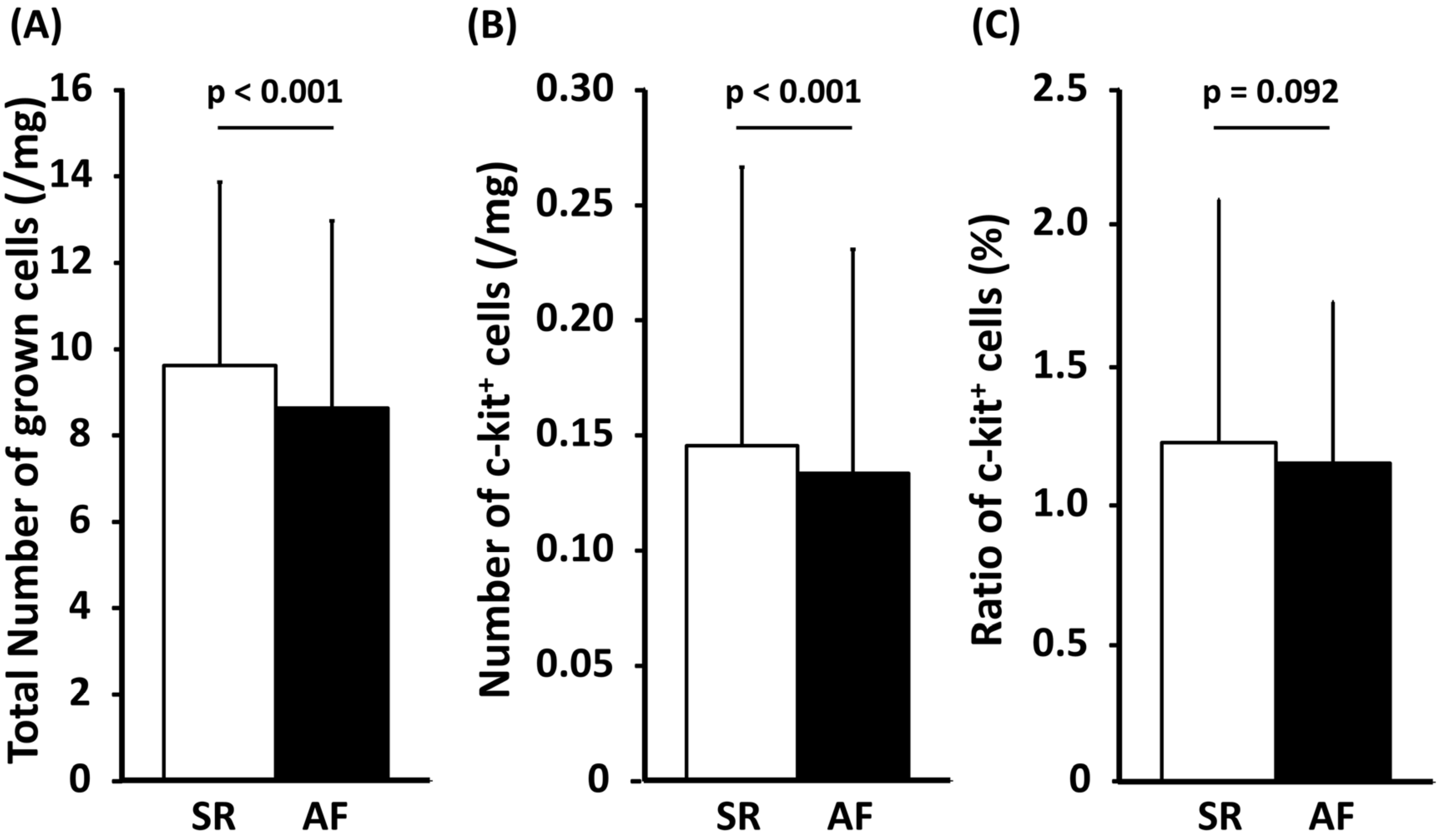
Total number of the outgrowth cells cultured from 1 mg of cardiac tissue was significantly lower from AF compared with SR (SR vs. AF: 9.8 ± 3.7 vs. 8.8 ± 4.2 [per mg], $p < 0.01$). The estimate number of c-kit positive cells (c-kit$^+$) was also significantly lower from AF (SR vs. AF: 0.15 ± 0.15 vs. 0.13 ± 0.10 [per mg], $p < 0.01$). The rate of c-kit positive cells contained in outgrowth cells was not different between groups (SR vs. AF: $1.18 \pm 1.04\%$ vs. $1.12 \pm 0.62\%$, $p = 0.34$).



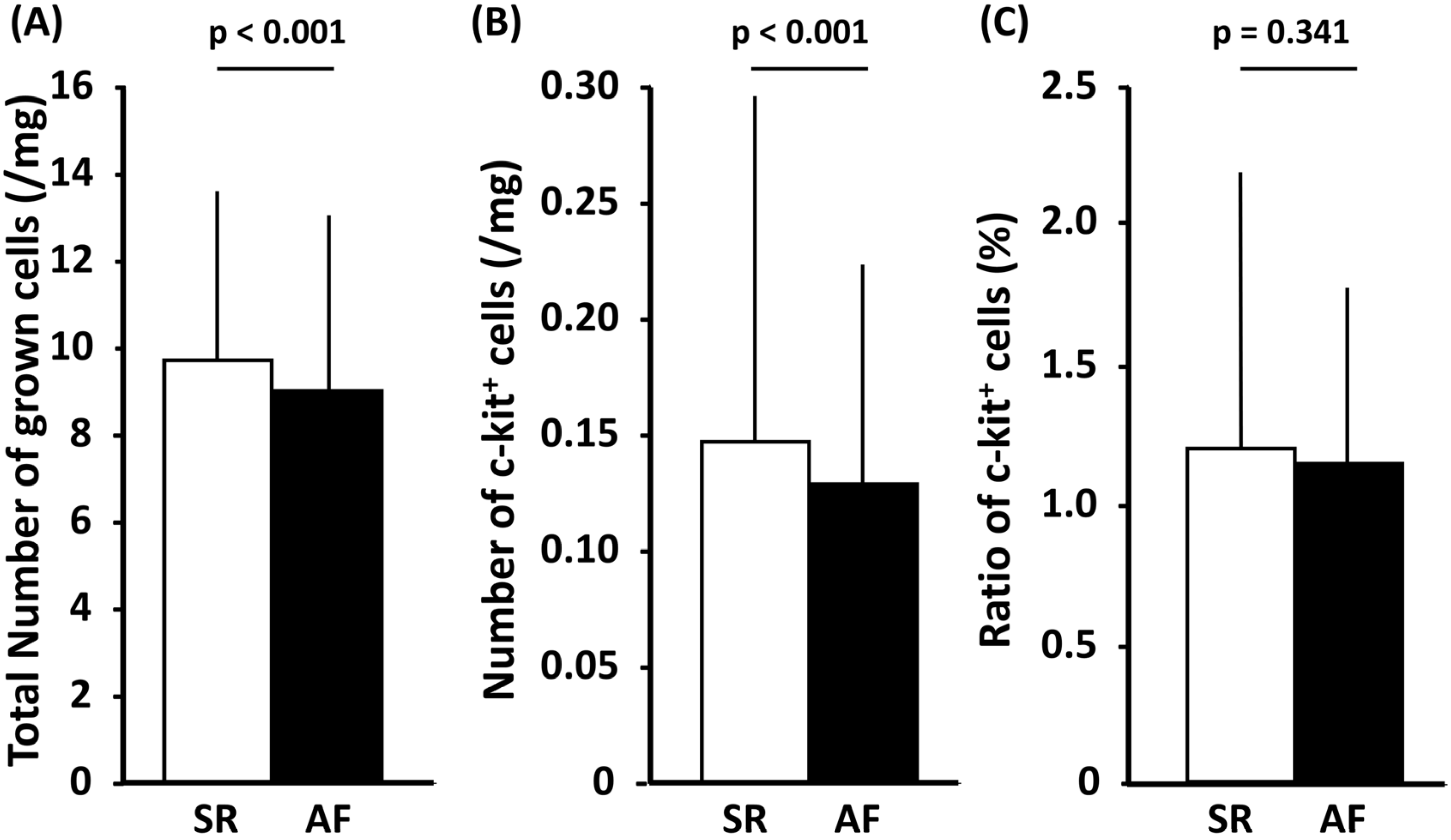
Relative mRNA expression vs. SR (-fold)
0
1
2
3
4
5
6
Sinus rhythm: SR
Atrial fibrillation: AF
**p < 0.01 vs. SR
**
**
**
**
**
Coll- I
bFGF
TGFβ1
TGFβ2
TGFβ3
ANP
BNP


(A) SR
(B) AF
(C)
p < 0.05
fibrotic area (%)
25
20
15
10
5
0
SR
AF


Relative mRNA expression vs. SR (-fold)
2.5
2.0
1.5
1.0
0.5
0
Sinus rhythm: SR
Atrial fibrillation: AF
**p < 0.01 vs. SR
**
**
Cx40
Cx43
Cx45



(A)
p < 0.001
Total Number of grown cells (/mg)
0
2
4
6
8
10
12
14
16
SR
AF
(B)
p < 0.001
Number of c-kit+ cells (/mg)
0
0.05
0.10
0.15
0.20
0.25
0.30
SR
AF
(C)
p = 0.092
Ratio of c-kit+ cells (%)
0
0.5
1.0
1.5
2.0
2.5
SR
AF



(A)
p < 0.001
Total Number of grown cells (/mg)
0
2
4
6
8
10
12
14
16
SR
AF
(B)
p < 0.001
Number of c-kit+ cells (/mg)
0
0.05
0.10
0.15
0.20
0.25
0.30
SR
AF
(C)
p = 0.341
Ratio of c-kit+ cells (%)
0
0.5
1.0
1.5
2.0
2.5
SR
AF

Table 1 Patients' characteristics among groups

	All	SR	AF	p value
N	319	214	105	
Female (%)	111 (34.8)	62 (29.0)	49 (46.7)	< 0.01
age, yr	65.9 ± 14.1	64.2 ± 15.7	69.6 ± 9.11	< 0.01
BMI	23.2 ± 3.2	23.1 ± 3.2	23.3 ± 3.3	0.60
HR, bpm	70.4 ± 12.6	70.0 ± 12.9	71.0 ± 12.0	0.43
HT	188 (58.9)	139 (60.3)	59 (56.2)	0.49
DM	72 (22.6)	50 (23.4)	22 (21.0)	0.62
DL	126 (39.5)	99 (46.3)	27 (25.7)	< 0.01
Smoking	158 (49.5)	108 (50.5)	50 (47.6)	0.63
Medication				
ACE/ARB (%)	114 (35.7)	76 (35.5)	38 (36.2)	0.90
Ca-blocker (%)	82 (25.7)	61 (28.5)	21 (20.0)	0.09
statin (%)	113 (35.4)	83 (38.8)	30 (28.6)	0.07
β-blocker (%)	111 (34.8)	70 (32.7)	41 (39.0)	0.27
BNP, pg/mL	183.8 ± 320.2	141.3 ± 316.9	260.5 ± 299.4	< 0.01
Cre, mg/dL	0.9 ± 0.4	0.8 ± 0.4	0.9 ± 0.4	< 0.01

T-chol, mg/dL	172.6 ± 37.2	170.7 ± 32.0	173.5 ± 40.0	0.49
TG, mg/dL	115.1 ± 70.0	120.1 ± 77.2	104.2 ± 50.4	<0.05
NYHA	1.7 ± 0.7	1.6 ± 0.7	1.9 ± 0.7	< 0.01
Surgical procedures				
CABG (%)	102 (32.0)	83 (38.8)	19 (18.1)	< 0.01
aortic valve (%)	111 (34.8)	76 (35.5)	35 (33.3)	0.70
mitral valve (%)	158 (49.5)	64 (29.9)	94 (89.5)	< 0.01
tricuspid valve (%)	70 (21.9)	5 (2.5)	65 (61.9)	< 0.01
aorta (%)	47 (14.7)	38 (17.8)	9 (8.6)	< 0.05
congenital (%)	5 (1.6)	2 (0.9)	3 (3.2)	0.27
tumor (%)	4 (1.3)	4 (1.9)	0 (0.0)	< 0.05
Maze (%)	78 (24.5)	0 (0.0)	78 (74.3)	< 0.01

AF: atrial fibrillation, ACE: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blockers, BMI: body mass index, BNP: brain natriuretic peptide, Ca-blocker: calcium blocker, Cre: serum creatinine, DL: dyslipidemia, DM: diabetes mellitus, HR: heart rate, HT: hypertension, IHD: ischemic heart disease, NYHA: New York Heart Association classification, T-chol: total cholesterol, TG: triglycerides

Table 2 Preoperative echo data

	SR	AF	p value
LVDd, mm	51.6 ± 8.9	52.0 ± 7.2	0.74
LVDs, mm	34.7 ± 10.4	34.8 ± 7.0	0.80
LAD, mm	38.9 ± 7.8	54.0 ± 10.0	< 0.01
LVEDV, mL	135.8 ± 62.1	132.3 ± 48.1	0.73
LVESV, mL	59.9 ± 46.0	59.3 ± 30.6	0.87
IVST, mm	10.4 ± 1.6	10.2 ± 1.3	0.92
PWT, mm	10.5 ± 3.3	10.2 ± 1.3	0.18
LVEF, %	59.0 ± 15.3	59.3 ± 12.1	0.99

IVST: interventricular septal wall thickness, LAD: left atrium diameter, LVDd: left ventricular end-diastolic diameter, LVDs: left ventricular end-systolic diameter, LVEDV: left ventricular end-diastolic diameter, LVESV: left ventricular end-systolic diameter, LVEF: left ventricular ejection fraction, PWT: posterior wall thickness

Table 3. Characteristics of the patients with mitral valve disease

	All	SR	AF	p value
N	158	64	94	
Female (%)	70 (44.3)	24 (37.5)	46 (48.9)	0.15
age, yr	64.7 ± 14.0	57.8 ± 16.9	69.4 ± 9.0	< 0.01
BMI	22.9 ± 3.3	22.7 ± 3.4	23.1 ± 3.2	0.44
HR, bpm	71.9 ± 11.3	71.8 ± 12.0	71.9 ± 10.8	0.96
HT	81 (51.3)	28 (43.8)	53 (56.4)	0.12
DM	24 (15.2)	6 (9.4)	18 (19.1)	0.08
DL	43 (27.2)	21 (32.8)	22 (23.4)	0.94
Smoking	70 (44.3)	27 (42.2)	43 (45.7)	0.61
Medication				
ACE/ARB (%)	53 (33.5)	17 (26.6)	36 (38.3)	0.13
Ca-blocker (%)	34 (21.5)	15 (23.4)	19 (20.2)	0.64
statin (%)	38 (24.1)	12 (18.8)	26 (27.7)	0.20
beta-blocker (%)	53 (33.5)	17 (26.6)	36 (38.3)	0.12
BNP, pg/mL	253.9 ± 410.0	216.6 ±512.0	275.9 ± 325.5	0.39
Cre, mg/dL	0.9 ± 0.4	0.8 ± 0.3	0.9 ± 0.4	< 0.01
T-chol, mg/dL	174.2 ±39.8	180.3 ± 47.9	170.1 ± 32.8	0.12
TG, mg/dL	114.9 ± 74.4	130.1 ± 97.3	103.9 ± 51.6	0.06
NYHA	1.8 ± 0.8	1.6 ± 0.8	2.0 ± 0.7	< 0.01
Surgical procedures				
CABG (%)	28 (17.7)	12 (18.8)	16 (17.0)	0.78
aortic valve (%)	41 (25.9)	11 (17.2)	30 (31.9)	< 0.05

tricuspid valve (%)	65 (41.1)	3 (4.7)	62 (66.6)	< 0.01
aorta (%)	14 (8.9)	7 (10.9)	7 (7.4)	0.46
congenital (%)	3 (1.9)	1 (1.6)	2 (2.1)	0.79
tumor (%)	1 (0.6)	4 (1.9)	0 (0.0)	0.23
Maze (%)	73 (46.2)	0 (0.0)	73 (77.7)	0.01
Echocardiogram data				
LVDd, mm	52.8 ± 6.8	55.5 ± 8.1	52.3 ± 7.2	< 0.05
LVDs, mm	34.9 ± 6.9	37.0 ± 10.5	34.9 ± 7.0	0.17
LAD, mm	52.7 ± 11.2	43.4 ± 7.7	55.0 ± 10.1	< 0.01
LVEDV, mL	135.1 ± 40.6	152.7 ± 50.2	134.3 ± 48.6	< 0.05
LVESV, mL	56.9 ± 27.1	63.2 ± 44.1	58.3 ± 31.1	0.45
IVST, mm	10.0 ± 1.1	10.2 ± 1.3	10.1 ± 1.3	0.62
PWT, mm	10.1 ± 1.3	10.1 ± 1.3	10.2 ± 1.3	0.73
LVEF, %	61.4 ± 10.8	59.9 ± 18.2	59.3 ± 12.3	0.86