Factors for C-Kit Expression in Cardiac Outgrowth Cells and Human Heart Tissue

Satoshi Matsushita,1 MD, Kazuo Minematsu,2,3 PhD, Taira Yamamoto,1 MD, Hirotaka Inaba,1 MD, Kenji Kuwaki,1 MD, Akie Shimada,1 MD, Yasutaka Yokoyama,1 MD and Atsushi Amano,1 MD

Summary

We determined the factors associated with the expression of c-kit in the heart and the proliferation of c-kit-positive (c-kitpos) cardiac stem cells among the outgrowth cells cultured from human cardiac explants. Samples of the right atrium (RA), left atrium (LA), and left ventricle obtained from patients during open-heart surgery were processed for cell culture of outgrowth cells and tissue analysis. The total number of growing cells and the population of c-kitpos cells were measured and compared with c-kit expression in native tissues and characteristics of the patients according to the region of the heart.

We analyzed 452 samples from 334 patients. Atrial fibrillation (AF) in the patients reduced the number of outgrowth cells from the RA and LA, and aging was a co-factor for the LA. The c-kitpos population from the RA was associated with serum brain natriuretic peptide (BNP). C-kit expression in native tissue was also associated with BNP expression. However, we observed no relationship in expression between outgrowth cells and native tissue. In addition, the RA tissue provided the highest number of c-kitpos cells, and the left ventricle provided the lowest.

C-kit was weakly expressed in response to damage. In addition, no correlation between outgrowth cells and native tissue was found for c-kit expression.

Key words: Cardiac stem cells, Heart injury, Self-repair, Brain natriuretic peptide, Aging, Atrial fibrillation

The adult human heart is no longer considered a terminally differentiated organ, but may have the ability of self-renewal. The heart contains resident stem cells that may play an important role in cardiac regeneration.11 Cardiac stem cells (CSCs) are an attractive candidate for a source of cell therapy for patients with heart injury, because CSCs are of heart origin,2 differentiate into cardiomyocytes, endothelial cells, or vascular cells,1,3 and may contribute to endogenous cardiac repair.12 A method to culture CSCs derived from adult heart tissue was established in 2004.5 In this method, outgrowing cells that are expanded from cardiac specimens include a population that is positive for c-kit, a stem cell marker. Although many other markers may determine CSCs,1,3,6,7 c-kit is the most widely used marker.

Some reports have demonstrated that the number of c-kit-positive (c-kitpos) CSCs is not only associated with age or sex, but also increases in response to heart injury.6-10 In contrast, a recent report showed that endogenous c-kit barely contributes to cardiac regeneration.11 Despite the vigorous discussion, the underlying mechanism and other potential factors involved in the expression of c-kit, and moreover, the characteristics of the c-kitpos cells in the native heart compared to cells isolated from cardiac tissue, are unclear. In addition, the percent of c-kitpos cells among outgrowth cells has varied among reports.3,9,12 Although an increase in the number of c-kitpos cells following heart injury has been reported in the native heart10 and in expanded cells, the correlation between the numbers of these cell types has not been elucidated.

An increasing number of patients have chronic heart failure, and stem cell therapy is expected to be a novel therapeutic option for severe heart failure. Thus, increased knowledge and detailed information about stem cells may suggest strategies for proper treatment for heart damage, including the therapeutic use of these cells. In this study, we aimed to determine the factors associated with the proliferation and c-kit expression of cardiac outgrowth cells using heart tissue obtained during open-heart surgery in a large number of patients. Furthermore, we also evaluated the relationship between the proportion of c-kitpos cells in the native heart and expanded cells.
Methods

Patients: Patients signed a written consent form according to a protocol approved by the Internal Review Committee on Ethics of Human Investigation of the Juntendo University Hospital prior to collection of the tissue sample. This study was performed in compliance with the Declaration of Helsinki. Patients who underwent open-heart surgery were enrolled in this study. Patients who had undergone open-heart surgery previously or who had received chest irradiation were excluded from this study.

Tissue collection and culture of cardiac outgrowth cells: The cardiac tissue was obtained during cardiac surgery. Immediately after dissecting tissues, the samples were immersed in cardioplegic solution (Miotecter®, Mochida Pharma, Tokyo, Japan), and then transferred to the laboratory. The sample was cut into small pieces, the epicardium and endocardium membrane was peeled off, and the sample was trimmed to a final weight of 20-30 mg for primary cell culture. Another piece of the sample, if sufficient tissue was available, was prepared for RNA isolation. The sample for culture was further 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 Medium (Life Technologies, Tokyo Japan) containing 10% fetal bovine serum (Thermo Scientific, Yokohama, Japan), 1% penicillin-streptomycin (Life Technologies, and 2 mM L-glutamine (Life Technologies). The medium was changed every 3-4 days. After 28 days of culture, a primary outgrowth of cells had grown out radially in a monolayer from the cardiac tissue, and these cells were harvested with 0.05% trypsin-EDTA and counted.

Flow cytometry: After harvesting the outgrowing cells, a portion of the cells was fixed with 4% paraformaldehyde (Sigma) for 15 minutes. The cells were then washed with PBS twice 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) for 2 hours at 4°C. The cells were then washed with PBS and analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Bioscience).

Reverse transcription-polymerase chain reaction (RT-PCR): mRNA was extracted from the heart tissue with standard laboratory techniques 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 HT-7700 (Life Technologies) and the SYBR-green kit (Life Technologies). Primers were designed to amplify and quantify GAPDH (forward: 5’-AT GGGGAAGTGAAGGTCG-3’; reverse: 5’-GGGTCAT TGATGGCAACAATA-3’) and c-kit (forward: 5’-GTCTCT GCTCCTACTGCTTCGC-3’; reverse: 5’-CCACGGCGGAC TATTAAGTCTGA-3’). The relative abundance of the target gene was obtained by calculating the amount of genespecific mRNA that was normalized to GAPDH as an internal control.

Statistical analyses: The Kolmogorov-Smirnov test was used to assess the normality of the data distribution before all statistical analyses were performed. Comparison of patients’ background among samples was performed using chi-square analysis and the Kruskal-Wallis test. Comparison of the properties of the outgrowth cells in all samples originating from the heart was examined using the Kruskal-Wallis test and Steel-Dwass test. The difference in properties of the outgrowth cells and mRNA expression between the left atrium (LA) and right atrium (RA) was determined with the Mann-Whitney U-test. The relationships between the expression of c-kit mRNA in native cardiac tissue and the total cell number of outgrowth cells, the rate of c-kit cells, and the number of c-kit cells were estimated with the Spearman’s rank correlation coefficient. The factors involved in the proliferation of the outgrowth cells and the population of c-kit cells derived from the LA and RA were investigated with stepwise multiple regression analysis. In the development data set, we formulated a predictive equation based on sex, age, body mass index, ischemic heart disease, treatment with cardio-pulmonary bypass, hypertension, diabetes mellitus, dyslipidemia, pre-operative hemodialysis, smoking (present/histor), persistent atrial fibrillation (AF), serum brain natriuretic peptide (BNP), classification of New York Heart Association (NYHA), diameter of the LA, and left ventricular ejection fraction (LVEF). Multicollinearity among the variables in the predictive equation was considered. Data were analyzed using SPSS 22.0 (SPSS, Tokyo, Japan). All data are the mean ± standard deviation. Statistical significance was set at a level of 0.05.

Results

Of the 452 registered patients, cardiac tissue samples were obtained from 364 patients and processed for cell culture. Among them, 10 cases were excluded due to re-sternotomy, and 14 cases were lost due to contamination during culture. In addition, six cases were discarded because of failure of cell growth. Finally, 452 samples from 334 patients were analyzed. In this study, because multiple tissues from different parts of the heart were obtained from some patients, the analyses were performed for individual regions of the heart. As a result, a single sample was obtained from 221 patients, and multiple samples were obtained from the other 113 patients (detailed information of the tissue origin is described in the Supplemental Table). The details of the clinical background of the patients are shown in Table I, and surgical procedures are listed in Table II. Of note, all patients who underwent isolated coronary artery bypass grafting (CABG) surgery, except for one with an on-pump beating technique because of low LVEF, received off-pump CABG surgery. Other CABG procedures with cardio-pulmonary bypass were concomitant procedures with valve or aortic surgery.

Factors that affect the characteristics of cardiac outgrowth cells: analysis according to region: Analyses of samples were performed for each region of the heart. After culture of outgrowth cells for 28 days, the total number of outgrowth cells (CN), the rate of c-kit cells (c-
kit\textsuperscript{\textbf{pos}_{R}}, and the total number of c-kit\textsuperscript{\textbf{pos}} cells (c-kit\textsuperscript{\textbf{pos}} \_N) were compared according to the background of the patients. The patients’ characteristics for each region of the heart are shown in Table III. The overall proportion of c-kit\textsuperscript{\textbf{pos}} cells in the outgrowth cells was 0.14-4.89\% (average, 1.18\%). For the cells derived from the LA (n = 291), age and prevalence of pre-operative AF were the factors that significantly reduced the CN (r = 0.216, P < 0.05). Aging was the only factor that significantly reduced the c-kit\textsuperscript{\textbf{pos}} \_N (r = 0.201, P < 0.05), and no factor affected the c-kit\textsuperscript{\textbf{pos}} \_R.

In contrast, for the outgrowth cells derived from the RA (n = 143), AF was the only factor that significantly reduced the CN (r = 0.244, P < 0.05), and pre-operative serum BNP significantly increased both the c-kit\textsuperscript{\textbf{pos}} \_R and c-kit\textsuperscript{\textbf{pos}} \_N (r = 0.321, P < 0.05 for c-kit\textsuperscript{\textbf{pos}} \_R and r = 0.503, P < 0.01 for c-kit\textsuperscript{\textbf{pos}} \_N). For the outgrowth cells derived from LV tissue, the number of samples (n = 18) was too small for statistical evaluation of the factors.

Comparison of the properties of the outgrowth cells according to the origin of the cells: We looked for the differences in the properties of the outgrowth cells according to the origin of the cells. First, all samples were compared according to whether the cells were derived from the LA, RA, or LV (Figure 1). For the CN, RA tissue provided the largest number of outgrowth cells followed by LA tissue (LA versus RA versus LV = 9.30 ± 4.30 versus 11.21 ± 5.90 versus 5.36 ± 3.40 [per mg], respectively, P < 0.01, for each comparison). The rate of c-kit\textsuperscript{\textbf{pos}} cells was significantly different between the RA and each of the other two regions (LA versus RA versus LV = 1.15 ± 0.76\% versus 1.29 ± 0.84\% versus 1.05 ± 0.95\%, respectively, P < 0.01 for LA versus RA and RA versus LV). The c-kit\textsuperscript{\textbf{pos}} \_N was the highest from RA tissue (LA versus RA versus LV = 1.21 ± 0.01 versus 1.76 ± 1.58 versus 0.57 ± 0.62 \times 10^{11} \text{per mg}, respectively, P < 0.01), similar to the CN.

In 102 cases, both LA and RA tissues were obtained from a single patient. To remove inter-patient variation, we then compared the properties of the cells between these samples (Figure 2). The CN obtained from RA tissue was significantly higher than that from LA tissue (LA versus RA; 9.16 ± 4.35 versus 10.65 ± 5.16, P < 0.01). Although the c-kit\textsuperscript{\textbf{pos}} \_R was not significantly different between the LA and RA (LA versus RA = 1.20 ± 0.71 versus 1.21 ± 0.76 \%, respectively, P = 0.815), the c-kit\textsuperscript{\textbf{pos}} \_N was significantly higher in RA tissue than LA tissue (LA versus RA = 1.33 ± 1.04 versus 1.69 ± 1.53, respectively, P = 0.232). In addition, the CN, c-kit\textsuperscript{\textbf{pos}} \_R, and c-kit\textsuperscript{\textbf{pos}} \_N tended to be low in cells cultured from LV tissue compared with atrial tissues in the same patients. However, the number of samples (n = 5) was too low to perform statistical analysis.

**Table 1. Patient Characteristics**

| Variable                  | n   | Value       |
|---------------------------|-----|-------------|
| Number of patients        | 334 |             |
| Age, years                | 65.8±14.4 |
| Female (%)                | 117 (35.0) |
| Body mass index           | 23.2±3.2 |
| Hypertension (%)          | 251 (75.1) |
| Diabetes mellitus (%)     | 68 (20.4) |
| Dyslipidemia (%)          | 178 (53.3) |
| Hemodialysis (%)          | 20 (6.0)  |
| Smoking (%)               | 146 (43.7) |
| Atrial fibrillation (%)   | 78 (23.4)  |
| Medication                |      |             |
| ACE/ARB (%)               | 153 (45.8) |
| Calcium-blocker (%)       | 105 (31.4) |
| Statin (%)                | 148 (44.3) |
| Beta-blocker (%)          | 123 (36.8) |

ACE indicates angiotensin-converting enzyme inhibitor; and ARB, angiotensin II receptor blockers.

**Table 2. Surgical Procedures**

| Procedure                  | n   |
|----------------------------|-----|
| Coronary artery bypass grafting | 104 |
| Off-pump (%)               | 65 (62.5) |
| Aortic valve               | 126 |
| Mitral valve               | 98  |
| Tricuspid valve            | 19  |
| Aorta                      | 32  |
| Congenital heart disease   | 6   |
| Maze procedure             | 35  |
| Left ventricular plasty    | 2   |
| Morrow procedure           | 14  |
| Others                     | 26  |
Table III. Comparison by Origin of Tissue Samples

|          | Left atrium | Right atrium | Left ventricle | P   |
|----------|-------------|--------------|----------------|-----|
| n        | 291         | 143          | 18             |     |
| Age, years | 64.7 ± 14.7 | 67.6 ± 14.3  | 74.6 ± 8.8    | <0.01|
| Female (%) | 98 (33.7)   | 50 (35.0)    | 11 (61.1)      | 0.06 |
| BMI      | 23.0 ± 3.2  | 23.4 ± 2.8   | 22.1 ± 3.1    | 0.32 |
| HT (%)   | 215 (73.9)  | 111 (77.6)   | 11 (61.1)      | 0.12 |
| DM (%)   | 62 (21.3)   | 25 (17.5)    | 3 (16.7)       | 0.61 |
| DL (%)   | 151 (51.9)  | 64 (44.8)    | 16 (88.9)      | <0.01|
| HD (%)   | 15 (5.2)    | 9 (6.3)      | 0 (0.0)        | 0.52 |
| Smoking (%) | 128 (44.4) | 66 (46.2)    | 7 (33.9)       | 0.81 |
| AF (%)   | 65 (22.3)   | 42 (29.4)    | 2 (11.1)       | <0.05|
| NYHA     | 1.7 ± 0.8   | 1.7 ± 0.8    | 2.1 ± 0.6      | 0.08 |
| BNP, pg/mL | 254.4 ± 551.1 | 245.3 ± 417.7 | 294.2 ± 450.1 | 0.07 |
| LVEF, %  | 59.4 ± 14.7 | 61.9 ± 11.9  | 71.1 ± 6.0    | <0.01|
| LAD, mm  | 42.8 ± 10.2 | 43.2 ± 10.8  | 43.1 ± 8.1    | 0.72 |
| IHD (%)  | 100 (34.4)  | 27 (18.9)    | 5 (27.8)       | <0.01|

AF indicates atrial fibrillation; BMI, body mass index; BNP, serum brain natriuretic peptide; DL, dyslipidemia; DM, diabetes mellitus; HD, Hemodialysis; HT, hypertension; IHD, ischemic heart disease; LVEF, left ventricular ejection fraction; LAD, left atrium diameter; and n, number of samples.

Figure 1. Comparison of properties of the outgrowth cells in all samples among the parts of the heart. The number of outgrowth cells (CN; A), rate of c-kitpos cells (c-kitpos_R; B), and total number of c-kitpos cells (c-kitpos_N; C) were compared among the parts of the heart. The CN cultured from RA tissue showed the highest value compared to the others, and that from the ventricle showed the lowest. We found no statistically significant differences in the c-kit pos_R among the tissues. The c-kitpos_N showed the same trend as the CN.

Discussion

We believe that this is the first study to examine the relationship regarding the expression of c-kit and the proliferation ability in native tissue and expanded cells. In addition, the factors affecting the properties of outgrowth cells in terms of the patients’ background were determined with the largest number of samples compared with previous studies.9,12,13) Another advantage of this study was the consistent inter-sample quality. Given that LA appendectomy is a standard procedure in our institution, a large portion of the LA appendage is obtained. A sufficiently large sample allowed cell culture with only the muscle layer after removal of the epicardial membranes. Furthermore, tissue analysis was performed with a piece of the same cardiac region that was used for the culture. Moreover, the ethnicity of the patients was very similar because all patients enrolled in this study, except for one who was mid-Asian, were of eastern Asian origin (mainly Japanese).

Factors associated with cell proliferation: Our results demonstrated that a common factor that reduced the total number of outgrowth cells (proliferation ability) was AF for both LA and RA tissues. AF is the most common type of arrhythmia and is strongly associated with inflammation14) and fibrotic remodeling of the tissue.15) Because the number of cells was adjusted according to tissue weight, tissue with a higher fibrotic content may produce a lower number of cells per weight.

Factors associated with the c-kitpos population: Many markers have been reported to specifically label CSCs (e.g., c-kit,1 Sca-1,7 or Isl-18). Among them, c-kit...
has been investigated the most and is the most common marker for identification of CSCs. In fact, in a clinical trial investigating ischemic heart disease, patients were administered CSCs sorted by c-kit expression.16) A previous report suggested that c-kitpos CSCs are activated when the heart is damaged.9) Although histological analysis of tissue injury was not performed in this study, the prevalence of ischemic heart disease, perioperative NYHA, and LVEF were not associated with the expression of c-kitpos cells in the LA or RA. In contrast, c-kitpos cells derived from RA tissue were associated with serum BNP, a general marker of heart failure.17,18) Secretion of BNP is increased in patients with congestive heart disease17,19) and is widely used as a sensitive marker of heart failure. In this study, the serum BNP level was the only detected factor that was associated with the expression of c-kit, although an increase in BNP is associated with aging, female gender, AF, and renal failure.20-23) Those data suggested that this endogenous hormone may play an important role in activation of the self-repair cascade. A recent study demonstrated that BNP stimulates the differentiation of cardiac progenitor cells into cardiomyocytes in vitro, and that injection of BNP into the mouse heart increases the number of newly formed cardiomyocytes.24) According to our results, serum BNP was the only factor that was associated with the increased expression of c-kit in native cardiac tissue and the c-kitpos population in RA-derived outgrowth cells. These results suggested that BNP may contribute to cardiogenesis by affecting cardiac progenitor cells.

**Figure 2.** Intra-patient comparison of properties of outgrowth cells between the left and right atrium. The number of outgrowth cells (CN), rate of c-kitpos cells (c-kitpos_R), and total number of c-kitpos cells (c-kitpos_N) were compared between the LA and RA of the same patients. The CN was significantly higher in the cells derived from the RA. Although the c-kitpos_R did not show significant differences, the c-kitpos_N was significantly higher in the cells from RA tissue.

**Figure 3.** Expression of c-kit in native tissue. The expression of c-kit mRNA was significantly higher in the native RA tissue than the native LA tissue.

**Figure 4.** Relationship between the serum BNP level and the expression level of c-kit in LA tissue. The expression of c-kit mRNA in LA tissue was compared based on the serum level of BNP [pg/dL]. The expression was significantly higher in the high-BNP group than in the low-BNP group.

The RA provides the most c-kitpos cells, both in native...
tissues and in expanded cells: Conflicting results have been reported regarding differences in stem cell activity among different regions of the heart. Some reports have suggested that the main source of CSCs is the atrium, especially the RA appendage; however, another study demonstrated the opposite result. When we compared the samples derived from the same patient, we observed that the number of c-kit cells was significantly higher in the RA than in LA. In addition, the number of cells from the ventricular tissue was the lowest. Interestingly, despite a difference in proliferation activity, the rate of c-kit cells in outgrowth cells was not different according to the origin of the samples. A potential reason may be differences in the oxygen concentration in the blood that passed through the tissues; the blood that passed through the RA had a much lower concentration of oxygen than the blood that passed through the LA. CSCs exist in a niche in which oxygen is low, implying better circumstances in RA tissue, especially on the endocardium side. Furthermore, the dominant transcription factors during embryonic stages are different between the atrium and ventricle, as well as between the right and left atrium. Further analysis of specific cardiac genes may identify genetic factor(s) that are associated with the properties of CSC. Another potential reason may be related to the prevalence of AF. Although the focus of AF varies, the most common origin is the pulmonary vein, which is located adjacent to the LA. Thus, tissue in close proximity to the AF focus may be affected by inflammation, resulting in reduced proliferation activity.

The role of CSCs: The most important question that still remains is: What is the role of CSCs? Although previous reports suggest that CSCs contribute to cardiac regeneration or repair, the myocyte renewal ability is insufficient for restoration of contractile function following injury. Additionally, if these cells play a role in cardiac repair, they should be activated in response to cardiac injury. However, our results demonstrated that most of the indicators of myocardial damage, surgery for ischemic heart disease, NYHA status, LVEF, and dilatation of the LA, did not affect the number of c-kit cells. Furthermore, our results demonstrated that the number of outgrowth cells as well as the c-kit population were significantly lower in cells isolated from LV tissue, the prominent region that causes circulatory failure.

Limitations: This study has some limitations. First, the patients’ backgrounds varied among the different cardiac samples, due to variations in the surgical procedures. Because LA appendectomy is a standard treatment option for patients who undergo open-heart surgery in our department, the highest number of samples obtained were from the LA. RA samples were obtained mainly from cases in which cannulas were inserted via the RA apex for extracorporeal circulation. However, we also perform off-pump techniques as standard procedures for isolated CABG, which does not require cannula insertion, and therefore, RA samples could not be obtained from such patients. Furthermore, the LV samples were obtained only from patients who had undergone septal myectomy due to ventricular hypertrophy or LV plasty for a ventricular aneu-
Disclosures

Conflicts of interest: The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114: 763-76.
2. White AJ, Smith RR, Matsushita S, et al. Intrinsically cardiac origin of human cardiospheres-derived cells. Eur Heart J 2013; 34: 68-75.
3. Smith RR, Barile L, Cho HC, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 2007; 115: 896-908.
4. Itzhaki-Alfia A, Leor J, Raanani E, et al. Patient characteristics and cell source determine the number of isolated human cardiac progenitor cells. Circulation 2009; 120: 2559-66.
5. Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 2004; 95: 911-21.
6. Laugwitz KL, Moretti A, Lam J, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 2005; 433: 647-53.
7. Matsushita K, Nagai T, Nishigaki N, et al. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. J Biol Chem 2004; 279: 11384-91.
8. Ellison GM, Vicinanza C, Smith AJ, et al. Adult c-kit+ cardiac stem cells are necessary and sufficient for functional cardiac regeneration and repair. Cell 2013; 154: 827-42.
9. Guo J, Jie W, Kuan D, et al. Ischaemia/reperfusion induced cardiac stem cell homing to the injured myocardium by stimulating stem cell factor expression via NF-kappaB pathway. Int J Exp Pathol 2009; 90: 355-64.
10. Franssio J, Bailey B, Gude NA, et al. Evolution of the c-kit-positive cell response to pathological challenge in the myocardium. Stem Cells 2008; 26: 1315-24.
11. van Berlo JH, Kanisicak O, Maillot M, et al. C-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014; 509: 337-41.
12. Leinonen JV, Emanuvelov AK, Plat Y, et al. Left atrial appendages from adult hearts contain a reservoir of diverse cardiac progenitor cells. PLoS One 2013; 8: e59228.
13. He JQ, Vu DM, Hunt G, Chuah A, Bhatnagar A, Bolli R. Human cardiac stem cells isolated from atrial appendages stably express c-kit. PLoS One 2011; 6: e27719.
14. Chung MK, Martin DO, Sprecher D, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. Circulation 2001; 104: 2886-91.
15. Goette A, Staack T, Ro¨cken C, et al. Increased expression of extracellular signal-regulated kinase and angiogenesis-converting enzyme in human atria during atrial fibrillation. J Am Coll Cardiol 2000; 35: 1669-77.
16. Bolli R, Chuah AR, D’Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase I trial. Lancet 2011; 378: 1847-57.
17. Maeda K, Tsutamoto T, Wada A, Hisanaga T, Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J 1998; 135: 825-32.
18. Anand IS, Fisher LD, Chiang YT, et al. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). Circulation 2003; 107: 1278-83.
19. Mukoyama M, Nakao K, Saito Y, et al. Increased human brain natriuretic peptide in congestive heart failure. N Engl J Med 1990; 323: 757-9.
20. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002; 40: 976-82.
21. McCullough PA, Duc P, Omland T, et al. B-type natriuretic peptide and renal function in the diagnosis of heart failure: an analysis from the Breathing Not Properly Multinational Study. Am J Kidney Dis 2003; 41: 571-9.
22. Yoshimura M, Yasue H, Morita E, et al. Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. Circulation 1991; 84: 1581-8.
23. Zhang Y, Chen A, Song L, Li M, Chen Y, He B. Association between baseline natriuretic peptides and atrial fibrillation recurrence after catheter ablation. Int Heart J 2016; 57: 183-9. (Review).
24. Bie1mann C, Rignault-Clerc S, Liaudet L, et al. Brain natriuretic peptide is able to stimulate cardiac progenitor cell proliferation and differentiation in murine hearts after birth. Basic Res Cardiol 2015; 110: 455.
25. Pouly J, Bruneval P, Mandet C, et al. Cardiac stem cells in the real world. J Thorac Cardiovasc Surg 2008; 135: 673-8.
26. Sanada F, Kim J, Czarna A, et al. c-Kit-positive cardiac stem cells nested in hypoxic niches are activated by stem cell factor reversing the aging myopathy. Circ Res 2014; 114: 41-55.
27. Jais P, Haïssaguerre M, Shah DC, et al. A focal source of atrial fibrillation treated by discrete radiofrequency ablation. Circulation 1997; 95: 572-6.
28. Kogawa R, Okumura Y, Watanabe I, et al. Difference between dormant conduction sites revealed by adenosine triphosphate provocation and unipolar pace-capture sites along the ablation line after pulmonary vein isolation. Int Heart J 2016; 57: 25-9.

Supplemental Files

Supplemental Table

Please see supplemental files; https://www.jstage.jst.co.jp/article/ihj/58/6/58_16-559/_article/supple