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Frizzled A, a novel angiogenic factor: promises for cardiac repair☆

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Abstract

Objective: Frizzled A is a very recent protein expressed in the cardiovascular hood by cardiomyocytes and by endothelial cells. This protein plays key roles in vitro in vascular cell proliferation and is able to induce an in vivo angiogenic response. Regarding these properties, we assess the hypothesis that Frizzled A could act in the healing process after myocardial infarction. **Methods**: To investigate the role of Frizzled A, we established a transgenic mouse line overexpressing the protein and developed a model of myocardial infarction by coronary artery ligation. **Results**: The incidence of cardiac rupture after myocardial infarction was reduced in transgenic mice (6.5 versus 26.4% in controls, $n = 165$; $P < 0.01$). Infarct sizes were smaller in transgenic mice (18% of left ventricle circumference versus 28.1% in control at day 30; $P < 0.001$; $n = 6$) and the cardiac function was improved (3800 ± 370 versus 2800 ± 840 mmHg/s dp/dt_{max} in controls, -2800 ± 440 versus -1800 ± 211 dp/dt_{min} in controls at day 15; $P < 0.001$; $n = 6$). Early leukocyte infiltration had decreased in transgenic mice during the first week (103 ± 59 versus 730 ± 463 cells/mm^2 in controls at day 7; $P < 0.001$; $n = 6$) and the apoptotic index was decreased by 50% at day 7. Capillary density in the scar was higher in transgenic mice (290 ± 103 versus 104 ± 43 vessels/mm^2 in control at day 15; $P < 0.001$) and vessels were more muscularized and mean lumen area was 3-fold higher (952 ± 902 versus 313 ± 350 μm^2 in control; $P < 0.001$). **Conclusion**: Overexpression of Frizzled A reduced the infarct size, improved cardiac recovery, modified inflammatory response and amplified angiogenesis. For these reasons, this protein would be of interest for cardiac surgeons using angiogenic therapy (as gene or protein injection) in ischemic heart diseases in non-revascularizable patients.

Keywords: Myocardial infarction; Angiogenesis; Inflammatory cell; Gene therapy

1. Introduction

Myocardial infarction is the most common cause of death in occidental countries. Infarct size is an important determinant of prognosis in humans. It has been known for many years that myocardial infarction induces prominent alterations of cardiac structure. Changes in the non-infarcted myocardium have been already described as well as cardiomyocyte hypertrophy and growth of capillary network. The modification of cardiac structure is the most determinant in cardiac function. Much pharmacological therapy (such as angiotensin converting enzyme inhibitors, blockade of the renin–angiotensin system) is used to modify the non-ischemic myocardium and demonstrate their effects in cardiac function improvement. However, recent studies point to the importance of the scar itself, suggesting that the myocardial healing process does not only result in dead tissue but is a very complex mechanism including at the cellular level apoptosis, proliferation, differentiation, cell migration and correct cell orientation to preserve an optimal cardiac function [1]. Briefly, the myocardial healing process after myocardial infarction can arbitrarily be divided into four phases: The first step is cardiomyocyte death essentially by apoptosis. The second step is characterized by an inflammatory response resulting in granulocyte infiltration in the scar in order to remove cell death. The third step is the adjustment of the granulation tissue corresponding to

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myofibroblast proliferation improving tensile strength. This phase is also characterized by the proliferation of many blood vessels. This angiogenic response increases tissue perfusion and improves cell survival. However, for unknown reasons, and this is the particularity of myocardial healing, a great part of vessels and fibroblasts disappears from the scar to leave a matrix rich in collagen: this is the last step also called the remodeling phase. Finally, the healing process after myocardial infarction is a very complex phenomenon.

In our laboratory, we work on a very recent protein, called Frizzled A (*Frizzled in Aorta*). This protein is implicated in a large system termed Wingless (Wnt)/Frizzled. Wnt are secreted protein comparable to multipotent growth factor and Frizzled are the corresponding receptors. The Wnt/Frizzled system is well known by embryologists and oncologists to play a role in embryonic development, in cell proliferation and in cell control polarity [2]. Recently, a new member of the Frizzled family has been described and termed 'secreted Frizzled Related Protein' (sFRP) [3]. sFRP proteins could antagonize the interaction between Wnt protein and Frizzled receptor. One of these, the protein Frizzled A (FrzA), was originally isolated from bovine aortic endothelium cells and characterized in our laboratory [4]. FrzA is related to human SARP2 (or human sFRP).

A growing body of evidence points to the involvement of Wnt/Frizzled in the cardiovascular system although their overall role is not known. Several studies demonstrate the impact of this system in cardiac morphogenesis [5], in atherosclerosis [6], in myocardial infarction [7], in myocardial hypertrophy [8] and recently in coronary heart disease [9]. Our laboratory previously detected high FrzA levels during cardiovascular maturation and in the cardiovascular system during adulthood [4,5]. FrzA was expressed by vascular cells and cardiomyocytes, and induced an angiogenic response [10].

Thus, as the Wnt-Frizzled system is involved in cardiac morphogenesis and in scar formation, as FrzA is expressed in heart and vessel and is involved in angiogenesis, we hypothesized that enhanced expression of FrzA might play a key role in cardiac healing after myocardial infarction. The aim of this study was to examine the effect of this secreted protein FrzA in transgenic mice in the healing process after myocardial infarction.

2. Materials and methods

2.1. Construction of transgenic mice

Overexpression of FrzA was realized under a CMV promoter in order to determine a ubiquitous pattern of expression. All experiments were performed with hemizygous mice for the transgene. For all experiments, we used transgenic mice backcrossed onto C57Bl6/J strain for more than ten generations in order to obtain purification. C57Bl6/J mice were used as controls. Genotypes were analyzed by polymerase chain reaction (PCR) amplification of specific sequences for FrzA cDNA from tail genomic DNA. The transgenic mice were viable, fertile and did not present any malformation in different organs observed by light microscopy.

2.2. Mice

All animals received humane care in compliance with the European Convention on Animal Care. This study was approved by the National Ethics Committee. Mice (transgenic and control) were kept in cages with access to food and water ad libitum until induction of anesthesia. For all experiments, males 9–12 weeks old and weighing 26–30 g were used.

2.3. Mouse model of myocardial infarction

Transgenic and control mice were anesthetized by a mixture of ketamine (2.5 ml/kg) and xylazine (0.8 ml/kg) via intraperitoneal injection. After induction, mice were orally intubated by a 22-gauge Cathlon catheter. They were then ventilated by a small animal respirator (Harvard Apparatus) with the following parameters: 0.06 ml for tidal volume and 120 breaths/min for rate. A left thoracotomy was performed in the fourth intercostal space. The ribs were retracted and the pericardium was opened. The left anterior descending coronary artery was tied by 7/0 silk polypropylene (Ethicon) near the tip of the left auricle. Ischemia was attested by the blanching and the dyskinesia of the left ventricle free wall and by the S-T segment elevation of the ECG. The chest was closed in three layers and the pneumothorax was exsufflated. After intraperitoneal rehydration and warm control temperature, mice were removed from the respirator.

2.4. Cardiac function analysis

Fifteen days after myocardial infarction, transgenic and control mice were anesthetized as described. A middle skin cervical incision was performed and the right carotid artery was cannulated by a 1.4-F Millar transducer (Millar). The aortic blood pressure was recorded and the catheter was pushed into the left ventricle: heart rate, left end-diastolic pressure, left end-systolic pressure, dp/dt_{max} and dp/dt_{min} without and with dobutamine infusion were recorded in a closed chest preparation.

2.5. Infarct size determination

Seven, 15 and 30 days after ligation, mice ($n = 6$), in each time and each group, were used for infarct size determination. Infarct size was performed by planimetry as described by Pfeffer et al. [11]. Mice were anesthetized as previously described. The thorax was opened by sternotomy and in rapid succession, the heart was arrested in diastole by 1 mEq KCl injection. The apex of the left

ventricle was cannulated with a 25-gauge catheter and a heparin phosphate buffer serum perfusion was started. The right auricle was cut to allow drainage and the pressure perfusion was adjusted to diastolic arterial pressure in order to avoid left ventricle cavity collapse or distention. The heart fixation was then realized in perfusion with a 4% paraformaldehyde (PFA) solution in the left ventricle apex. The heart was excised and stored for 3 h in 4% PFA. Two sections perpendicular to the longitudinal axis of the ventricle were performed. The three fragments (apex, intermedium, base) were embedded in paraffin and cut into serial 7-μm sections. The slides, mounted and stained by Masson's trichrome, were recorded with a CCD camera (Nikon, SMZ 2 T × 1.5 magnification) connected to an IBM PC. In the three sites examined, infarcted area, total left ventricle area, left ventricle cavity area, and thickness and length of the scar and the interventricular septum were recorded and averaged.

2.6. FrzA expression and localization after myocardial infarction

FrzA expression was only investigated in control mice by immunohistochemistry. To detect FrzA expression after myocardial infarction, sections were stained (2 days, 7 days and 15 days after ligation) with polyclonal antibody against FrzA as previously described [4].

2.7. Healing process analysis

The different steps of the healing process after myocardial infarction (days 2, 4, 7, 15), including apoptosis, inflammatory response, matrix lysis activity, angiogenesis, cell proliferation, cell differentiation, and collagen deposition (remodeling) were analyzed and compared in transgenic and control mice. For immunohistochemistry, mice were killed by lethal injection of sodium pentobarbital, the hearts were excised and fixed overnight in methanol. Experiments were performed as previously described [12]. A minimum of five animals in each group and at each time point after ligation were examined. To detect apoptosis in the scar, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining (TUNEL) was used. Nuclei were considered apoptotic by the presence of dark brown staining. To determine inflammatory response, myeloperoxidase staining (Myeloperoxidase, Dako), T-lymphocytes (CD-3, Serotec) and macrophages (F4/80, Caltag) were used. To analyze angiogenic activity, endothelial cells were stained with CD-31 (Pharmingen) antibody and smooth muscle cells were stained with α-actin antibody (Sigma). Cell proliferation was determined by BrdU incorporation (thymidine analogue, Harlan). Collagen deposition was stained with 0.1% Sirius red solution. Matrix lysis activity was determined by gelatin zymography: control and transgenic hearts ($n = 3$ in each group) were frozen after myocardial infarction to study MMP-2 and -9 activity. The scar after myocardial infarction (day 4 and day 7) was harvested. Tissue samples were homogenized in 500 μl of buffer. Samples were applied to non-denaturing 10% polyacrylamide gels containing 1 mg/ml gelatin. Following electrophoresis, the gels were washed with 2.5% Triton X-100, and incubated overnight at 37 °C in zymography buffer and stained with Coomassie Brilliant Blue. Gelatinolytic activity was visualized as clear areas of lysis in the gel.

2.8. Data analysis

Infarct size was determined as the percentage of left ventricle necrosis/left ventricle area. For immunohistochemistry analysis, a minimum of 30 randomized pictures were recorded at 40× magnification for each animal with a CCD camera connected to an IBM PC. Positive cells were manually counted with the help of Sigma Scan Plot software. Apoptosis, inflammatory cells, capillary density, and cell proliferation in the scar were reported per mm^2.

2.9. Statistical analysis

Results are expressed as mean ± SD. All analyses were performed with appropriate software (Stat-View 5-1). Comparisons of continuous variables between two groups were performed by one-way analysis of variance and if statistical significance was observed, a two-sided paired *t*-test was realized. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Time course of FrzA expression after myocardial infarction

FrzA expression after coronary ligation in control mice was detectable at day 2 with immunohistochemistry at low levels in ischemic myocardium. At day 7, abundant FrzA was detected in the border ischemic zone (Fig. 1a,b); then at day 15, expression returned to near baseline level. Only few cells were stained in the scar itself.

3.2. FrzA reduces the incidence of cardiac rupture after infarction

After myocardial infarction, there was no difference in early mortality (within 24 h after surgery) between the two groups ($n = 165$) (Table 1). Between days 4 and 6 after myocardial infarction, 26.4% of control mice suffered fatal cardiac rupture of the left ventricle free wall (Fig. 2a). In contrast, only 6.5% of transgenic mice died by this mechanism ($P < 0.01$, Table 1).

3.3. FrzA improves cardiac function after infarction

Before myocardial infarction, there was no difference in blood pressure, heart rate and left ventricle pressure with or

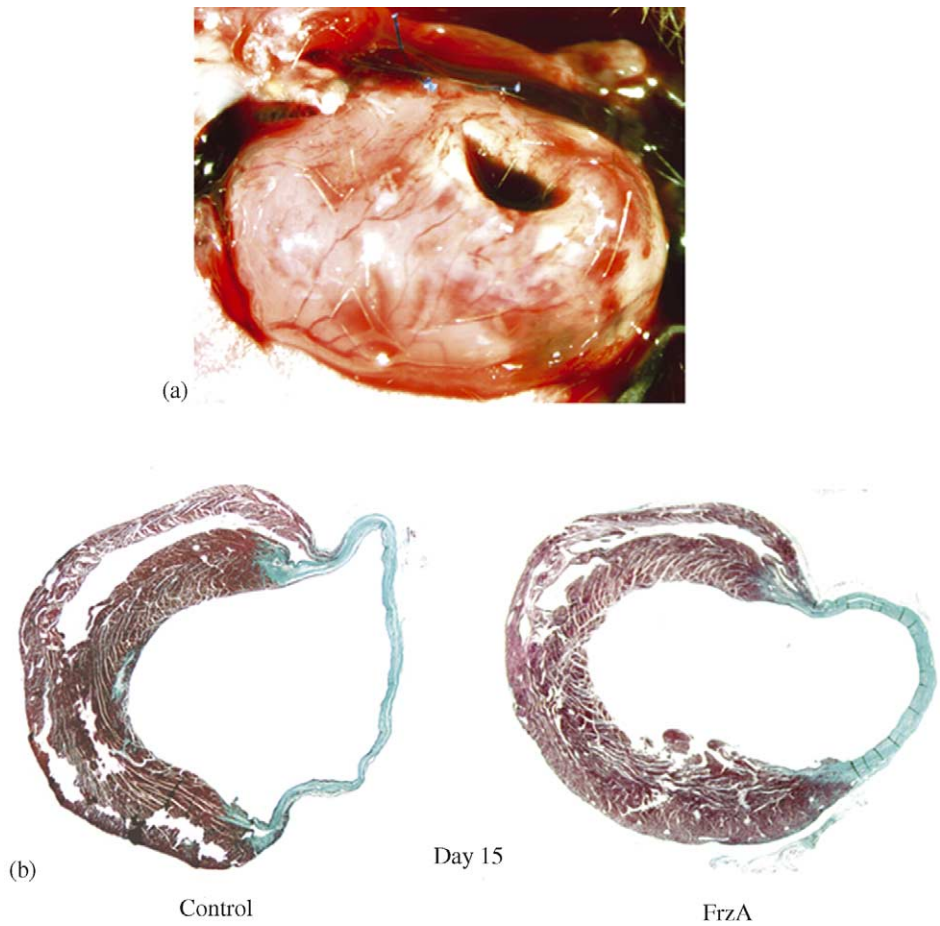

Fig. 1. (a) Cardiac rupture in control mice. Cardiac rupture was observed within days 4–6 after myocardial infarction. Near 26.4% of control mice suffered this mechanism of death in contrast to only 6.5% in transgenic mice. (b) Masson's trichrome staining 15 days after ligation. Hearts were arrested in diastole by 1 mEq KCl. The fixation was performed in perfusion with 4% PFA. Hearts were then excised and embedded in paraffin. Sections were stained with Masson's trichrome. Infarct size was smaller in transgenic mice overexpressing FrzA and the scar's thickness was increased as compared to control.

without dobutamine between the two groups of mice (Table 2). On day 15 after myocardial infarction, left ventricle end-diastolic pressure was significantly decreased in transgenic as compared to control mice (11 vs. 13 mmHg, $P < 0.01$). Left end-systolic pressure was significantly increased in transgenic mice as compared to control mice (80 vs. 90 mmHg, $P < 0.01$).

At day 15, the contractile index dp/dt_{max} was significantly increased and dp/dt_{min} significantly decreased in transgenic mice compared to control mice (Table 2, $P < 0.001$). Differences in dp/dt_{max} and dp/dt_{min} were confirmed and amplified after dobutamine infusion (Table 2, $P < 0.001$).

3.4. FrzA reduces infarct size

Before myocardial infarction, control and transgenic hearts displayed no difference in septal and free wall thickness and left ventricle dimensions. At day 7, day 15 and day 30 after myocardial infarction, the percentage of infarcted left ventricle was significantly decreased in transgenic mice compared to controls ($n = 6$; $P < 0.01$) Fig. 2b and Table 3). Moreover, the thickness of the scar was significantly increased in transgenic mice as compared to control mice at day 15 and day 30 ($n = 6$, $P < 0.001$) (Table 3 and Fig. 2b).

3.5. FrzA modifies the healing process

3.5.1. Inflammatory response

FrzA reduced leukocyte infiltration and apoptosis in the scar At day 2 and day 7 after ligation; transgenic mice displayed significantly less myeloperoxidase-positive cells

Table 1
Incidence of cardiac rupture

	Control	FrzA
Total operated ($n = 165$)	97	68
Early mortality (<24 h after surgery)	10 (10.3%)	7 (10.3%)
Differed mortality (day 4–6 cardiac rupture)	23 (26.4%)	4 (6.5%)*

Early and differed mortality after surgery. Cardiac rupture was assessed by necropsy. *$P < 0.01$, compared with control.

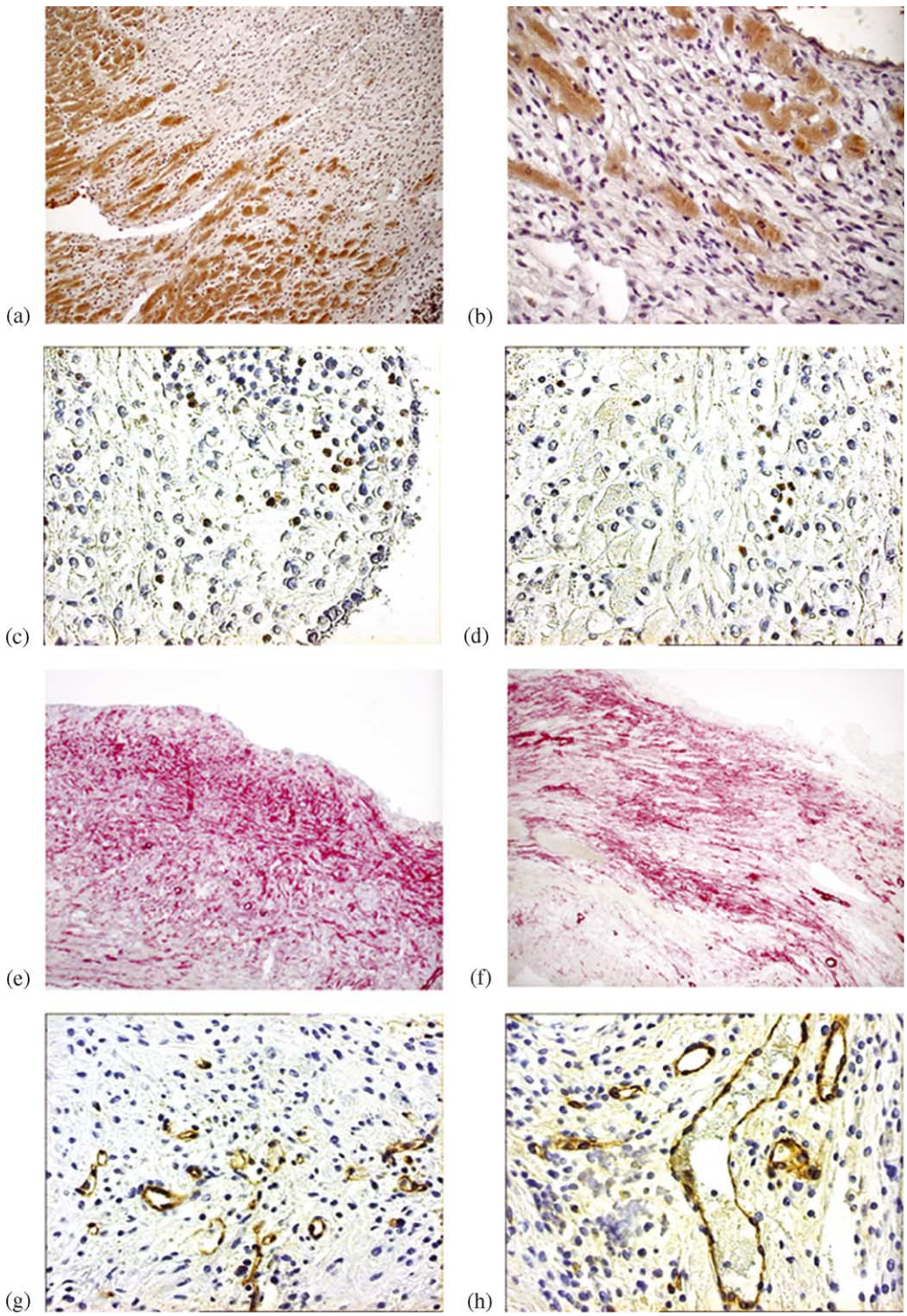

Fig. 2. (a) FrzA staining (× 15 magnification), 7 days after ligation in control mice. Note the overexpression of FrzA in the ischemic border zone. (b) FrzA staining (× 40), 7 days after myocardial infarction in control mice. Only a few cells expressed the protein in the scar itself. (c,d) Myeloperoxidase staining 7 days after ligation (× 40). The staining was less important in transgenic mice (d) as compared to control mice (c). (d,e) Smooth muscle cell α-actin staining, 7 days after myocardial infarction (× 20). Myofibroblasts colonized the scar. Note the difference in myofibroblast organization: in control mice (e); myofibroblasts were not in alignment but in contrast, in transgenic mice (f), myofibroblasts displayed a more concentric disposition, demonstrating the importance of cell polarity control in the scar for myocardial repair. (g,h) Angiogenesis in the scar: endothelial cell-specific immunostaining by CD-31 antibody 15 days after myocardial infarction (× 40). Capillary density and mean surface of vessels were most important in transgenic mice (h) as compared to control mice (g).

Table 2
Hemodynamics studies after myocardial infarction

	Without dobutamine infusion				With Dobutamine infusion			
	Control		FrzA		Control		FrzA	
Day post infarction	0	15	0	15	0	15	0	15
n	5	6	5	6	5	6	5	6
Heart rate (beats/min)	220 ± 8	227 ± 5	215 ± 2	220 ± 13	436 ± 10	390 ± 20	430 ± 16	400 ± 17
Systolic blood pressure (mmHg)	108 ± 4	83 ± 2	109 ± 1	94 ± 6*	188 ± 20	91 ± 9	165 ± 11	120 ± 13**
dp/dt_{max} (mmHg/s)	4500 ± 150	2800 ± 840	7700 ± 250	3800 ± 370**	4300 ± 200	4376 ± 339	7500 ± 180	6509 ± 845 **
dp/dt_{min} (mmHg/s)	−3800 ± 80	−1800 ± 211	−3700 ± 60	−2800 ± 440**	−9000 ± 70	−3146 ± 364	−8000 ± 50	−4240 ± 444**

Values are mean ± SD. *$P < 0.01$, **$P < 0.001$ compared with control on the same postsurgical day.

in the scar compared to control mice (1343 ± 302 cells/mm^2 in control vs. 856 ± 230 cells/mm^2 in transgenic mice at day 2 and 730 ± 463 cells/mm^2 in control vs. 103 ± 59 cells/mm^2 in transgenic mice at day 7; $P < 0.001$) (Fig. 1c, d). No difference was observed in macrophage (F4/80) staining (7 cells/mm^2 in control vs. 6 cells/mm^2 in transgenic mice at day 2 and 4 cells/mm^2 in control vs. 5 cells/mm^2 in transgenic mice at day 7; $P =$ NS (not significant)) and lymphocyte (CD-3) staining (22 cells/mm^2 in control vs. 22 cells/mm^2 in transgenic at day 2 and 10 cells/mm^2 in control vs. 11 cells/mm^2 in transgenic at day 7; $P =$ NS) in the scar between the two groups. At day 7, the total number of apoptotic cells was significantly decreased by 50% in transgenic mice compared to control mice (96 ± 14 cells/mm^2 in control vs. 45 ± 6 cells/mm^2 in transgenic mice; $P < 0.001$).

3.5.2. Matrix degradation and synthesis

FrzA decreased metalloprotease activity and increased collagen deposition. MMP activity was studied by gelatin zymography and showed a large decrease in MMP-9 activity and a moderate decrease in MMP-2 activity in the scar at day 4 in transgenic mice compared to control (not shown). These results were confirmed with MMP-9 immunostaining. Collagen density was significantly increased in the scar of transgenic mice compared to controls at day 15 (53 ± 2.3% of the scar area was Sirius red-positive in transgenic vs. 26 ± 6% in controls, $P < 0.001$).

3.5.3. Cellularity in the scar

FrzA improved cellularity and myofibroblast organization. Cell proliferation, as demonstrated by BrdU staining, was significantly higher in the scar of transgenic mice at day 15 after ligation compared to control mice (67 ± 51 cells/mm^2 in control vs. 135 ± 76 cells/mm^2 in transgenic; $P < 0.001$). Cell density at day 15 was twofold thicker in the transgenic scar as compared to control (4002 ± 496 vs. 2075 ± 359 cells/mm^2, $P < 0.001$). In transgenic mice, the cellular population was composed essentially of myofibroblasts, as assessed by α-actin staining. Moreover, at day 15, transgenic mice displayed a more concentric organization of collagen fiber and myofibroblasts (Fig. 1e,f).

3.5.4. Angiogenesis

FrzA improved angiogenesis in the scar. Coronary distribution, capillary density and area vessel muscularization were similar in normal uninjured heart in both groups. At day15, the capillary density was increased in transgenic mice as compared to control mice (290 vessels/mm^2 for transgenic versus 104 vessels/mm^2 for control mice at day 15, $P < 0.01$). These vessels were more muscularized in transgenic mice (67 ± 10% in transgenic vs. 50 ± 15% in control mice at day 15, $P < 0.01$). Moreover, the lumen area of capillaries was threefold greater in transgenic mice at day 7 and day 15 compared to control (952 μm^2 in transgenic versus 313 μm^2 in control mice at day15; $P < 0.001$, Fig. 1g,h). In conclusion, in the infarct scar capillary density,

Table 3
Morphometric analysis after myocardial infarction by ligation

	Control				FrzA			
Day post infarction	0	7	15	30	0	7	15	30
n	5	6	6	6	5	6	6	6
Body weight (g)	28	28.5	29.75	32	28	28	28.5*	29**
Infarct size (%)	–	65.4	44.4	28.1	–	28**	18*	18*
Infarct Thickness (mm)	0.8	0.6	0.36	0.33	0.9	0.8*	0.73**	0.57**

Infarct size is % of left ventricle necrosis/total left ventricle area. Values are mean ± SD. *$P < 0.01$, **$P < 0.001$ compared with control on the same postsurgical day.

vessel muscularization and lumen area of capillaries were greater in transgenic mice than in control mice.

4. Discussion

In this work, we have analyzed each phase of the myocardial healing process after myocardial infarction in mice. The time course of cardiac wound is strictly the same in humans with the only difference being in healing kinetics. In rodents such as mice or rats, the process is accelerated compared to humans. Moreover, work with mice offers the possibility to modify gene expression in order to study the effect of the candidate gene in a pathophysiological process [13]. Our strategy is based on an overexpression of FrzA. Considering FrzA expression and function, we have decided to explore its effect in myocardial repair after myocardial infarction. The cardiac phenotype of the transgenic mouse has been found to be normal, but following myocardial infarction, cardiac rupture and infarct size are reduced and cardiac function is improved. FrzA is capable of interfering in each step of myocardial repair. We demonstrate that FrzA prevents death of cardiomyocytes. The reduction of apoptosis and the limitation of inflammatory cell colonization in the scar are probably the crucial role of this protein. The modulation of the inflammatory response, as demonstrated by the reduction of leukocyte infiltration, improves cell survival [14], reduces MMP activity [15], and consequently decreases matrix lysis activity [16]. FrzA-induced MMP inhibition is accompanied by a decrease in cardiac rupture at day 4, as suggested previously [17], and a reduction in infarct wall thinning [16]. Cellularity in the infarcted area is increased in FrzA mice and most of these cells are myofibroblasts (as confirmed by α-actin staining). In parallel, we have found that myofibroblasts and collagen deposition in the transgenic heart are in alignment with the epi- and endocardium. These data confirm the importance of architectural and polarity cell control after myocardial infarction [7] to maintain cardiac function. Overexpression of FrzA increases capillary density, lumen area and muscularization in the scar. In fact, FrzA appears to preserve already formed capillaries at 7 days more than to increase vessel formation despite reduction of matrix lysis activity. Angiogenesis and vessel stabilization in ischemic heart disease are known to be essential for the improvement of tissue perfusion increasing cardiac function [18]. The angiogenic properties of FrzA could be compared to other angiogenic factors such as vascular endothelial growth factor, hypoxia-inducible factor-1α, or basic fibroblast growth factor. These angiogenic factors are experimentally able to improve capillary density and scar perfusion [19,20]. These data have been confirmed in clinical trials using angiogenic therapy, resulting in restoration of cardiac function [21,22]. As FrzA is a secreted protein and if our results are confirmed in other pre-clinical studies (pig or others), it would be interesting to realize FrzA injection (as recombinant protein or gene injection) in non-revascularizable ischemic heart disease. Biological bypass is now a new field of therapy offered by cardiac surgeons [23–25]. The exact mechanism of FrzA in some of these steps needs further investigation and the receptor is not yet known. Interestingly, we have recently observed after myocardial infarction in humans an overexpression of the FrzA human homologous (SARP-2), suggesting the interest of our report. These present findings suggest that FrzA would be a new target in myocardial repair after myocardial infarction.

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Appendix A. Conference discussion

Dr N. Tran (*Nancy, France*): I have many questions. First is about the concept of transplantation. You put the bone marrow cell back in the rat by intravenous injection. Do you know exactly the number of bone marrow cells could go back to these mice? Because we did the similar intravenous injection and there is retention in the lungs. And after that you can find it in the liver, and almost no portion of them that go back.

Dr Barandon: Very interesting question. But in fact, we do not use this bone marrow cells model in order to repopulate the scar. We only use bone marrow cells in order to express, only in these cell types, our transgene FrzA. So in fact, we have back-crossed our transgenic mice with ROSA 26 mice expressing beta-galactosidase activity. And we can study the chimerism of our mice by FACS analysis, and near 70% of our bone marrow cells after transplantation overexpressed FrzA.

We have studied by immunostaining, beta-gal staining, the repopulation of the scar itself. And interestingly, 15 days and 1 month after myocardial infarction, we are able to detect only near 1 to 2% of transplanted cell of the totality cellularity in the scar.

Dr Tran: I'm sorry, but I don't think so. When you induce myocardial infarction, the concept of cell therapy is that something, some mechanism, signal mechanism recruits bone marrow stem cells from peripheral system into the heart. So in your experiments, I would like to know if you did measure cells derived from bone marrow that they expressed your FrzA gene, firstly, and to know exactly if mesenchymal stem cells and prenatal stem cells go back to the heart to repair the heart or circulating cells in the blood can do it also?

Dr Barandon: We did not determine what cell type overexpressed FrzA in our bone marrow cell. Our mice were total body irradiated, and we transplant all bone marrow cells, not especially of cell type, CD34 or other.

But after myocardial infarction, as you certainly know, there is an important inflammatory response in order to remove the cell death, cardiomyocyte death. But, in fact, if we realize 2 days, 7 days, 15 days, and 1 month after a myocardial infarction, immunostaining, with X-gal staining or with beta-gal staining, we show that this is not bone marrow cell in the scar but it's only inflammatory cells. And it is very difficult to demonstrate, at this time, any transdifferentiation. There is an important recruitment by cytokines, TNF or interleukin 1, 6, or others, but their transdifferentiation was very low.