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TRANSPLANTATION OF SKELETAL MYOBLAST IN ISCHEMIC HEART DISEASE

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Declaration

I declare that the research presented in this thesis, including research design, data collection, and data analysis was conducted by the author, Guo Changfa. The results of this work have not been submitted for degree at any other tertiary institute.

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Guo Changfa

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Summary

Cell-based cardiac repair represents a promising therapeutic approach to treat heart failure. Among various cell types, skeletal myoblast (SkM) has been extensively used for cardiac cell therapy due to its myogenic potential, proliferative capacity, resistance to ischemia, and non-tumorigenic nature. The present study was to investigate the characteristics of human SkMs in vitro and in vivo, to investigate and compare immune responses, SkM survival profile, and SkM transplantation efficacy following xenogeneic, allogeneic, and autologous transplantation of SkMs in a rat myocardial infarction model.

By immunostaining and cell counting, we showed that immunocytes infiltrated severely in the early stage (from day-1 to day-7) after SkM transplantation. Macrophages and CD8+ lymphocytes infiltrated from day-1; CD4+ lymphocytes infiltrated from day-4, but all immunocytes subsided by day-28. By immunostaining, real time PCR, and β-gal assay, we confirmed and quantified the survival of SkMs. After transplantation, the majority of the SkM signals were rapidly lost by day-1. After day-1, a gradual increase in the number of SkMs was observed until 4 weeks after cell transplantation, resulting from the SkM proliferation out-balancing the gradual loss. One interesting finding of our study is that the grafted human SkMs and rat SkMs survive and differentiate well in the immunocompetent hosts even without any immunosuppression. From this we suggest that SkMs enjoy a non-autologous graft acceptance in myocardium, a finding which may have far reaching implications in clinical perspective. In addition, we demonstrated that there was a close correlation between immunocyte number and SkM total number.

In all SkM transplantation groups, SkM transplantation improved the heart performance by increasing the contraction function (ejection function) and limiting the ventricular dilation (left ventricular end diastolic diameter). Furthermore, we demonstrated that there was a linear relationship between the SkM survival and ventricular function as well. In our study, cyclosporine inhibited infiltration of the immune cells, enhanced the survival of transplanted SkMs and improved heart performance. Even in autologous groups, cyclosporine does enhance the heart performance.

This study enabled us a better understanding of the early cellular behavior of SkMs, especially human SkMs, and the underlying mechanisms that govern early graft attrition in SkM transplantation. The present study also suggests a feasibility of non-autologous SkM transplantation, especially allogeneic SkM transplantation.

Abbreviation

ABCG2+	ATP-binding cassette transporter
Ad	Adenovirus
AF	Atrial fibrillation
AMI	Acute myocardial infarction
BM	Bone marrow
BMCs	Bone marrow derived stem cells
BrdU	5-bromo-2'-deoxy-uridine
BSA	Bovine serum albumin
CABG	Coronary artery bypass grafting
CHF	Congestive heart failure
c-kit	Receptor for the stem cell factor.
CM	Cardiomyocyte
CSCs	Cardiac stem/progenitor cells
CX	Circumflex coronary artery
DAB	3, 3-diaminobenzidine
DAPI	4, 6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
ECG	Electrocardiogram
EF	Ejection fraction
ELISA	Enzyme linked immunosorbent assay
EPCs	Endothelial progenitor cells
Fb	Fibroblast
FBS	Fetal bovine serum
FITC	Fluorescein isothiocynate
FS	Fractional shortening
G-CSF	Granulocyte-colony stimulating factor
HRP	Horse radish peroxidase
HSCs	Hematopoietic stem cells
hSkM	Human skeletal myoblast
IC	Introcoronary infusion
ICS	Intra coronary sinus
IHD	Ischemic heart disease
Isl-1+	Insulin gene enhancer binding protein
KDR/Flk-1+	Vascular endothelial growth factor receptor
LAD	Left anterior descending artery.
Lin	Lineage markers
LVAD	Left ventricular assist device
LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume
MDR1+	P-glycoprotein
MI	Myocardial infarction
MMLV	Moloney Murine Leukemia Virus
MSCs	Mesenchymal stem cells
FISH	Fluorescence in situ hybridization

HPF	High power field
LVEDD	Left ventricular end diastolic diameter
MHC	Major histocompatibility complex
NYHA	New York Heart Association
OD	Optical density
PBS	Phosphate buffered saline
PCI	Percutaneous coronary intervention
PEI	Percutaneous endoventricular injection
PET	Positron emission tomography
rSkM	Rat skeletal myoblast
Sca-1	Stem cell antigen 1
SkM	Skeletal myoblast
SMA	Smooth muscle actin
SP	Cardiac side population
SSEA-1	Stem cell marker stage-specific embryonic antigen 1.
UPCs	Uncommitted cardiac precursor cells.
VT	Ventricular tachycardia
X-gal	5-bromo-4-chloro-3indoyl-β-D-galactosidase

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Publications

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Chapter Two: Materials and Methods