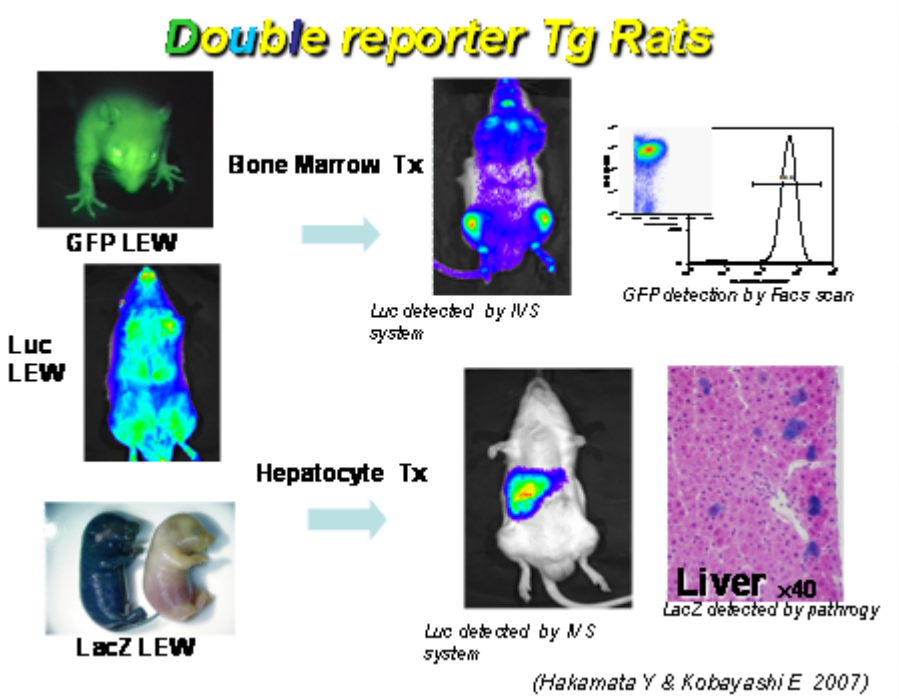
The Power of Bio-imaging Rat! The Research Results with Tokyo Medical and Dental University

Mice as one of the experimental animals have been widely used as a superb model. On the contrary, due to the physical body size of rats; 10 times bigger than mice, despite the fact that they are more suitable research tool for regenerative medicine, the establishment of experimental rats has been affected by the delay in genetic manipulation technology and cost factors. Prof. Eiji Kobayashi M.D., Ph.D. pushed forward the world-first research on genetic modification in the early 2000s by establishing bioimaging rats through the optimization of inbred rat species. The research group led by Prof. Ichiro Sekiya of Tokyo Medical and Dental University this time has achieved remarkable results on regeneration of meniscus and tendon grafts by the use of his bioimaging rats and has been published in STEM CELLS 2015;33:1927–1938.

Although meniscus defects and degeneration are strongly correlated with the later development of osteoarthritis, the promise of regenerative medicine strategies is to prevent and/or delay the disease's progression. Meniscal reconstruction has been shown in animal models with tendon grafting and transplantation of mesenchymal stem cells (MSCs); however, these procedures have not shown the same efficacy in clinical studies.

Here, the research was to investigate the ability of tendon grafts pretreated with exogenous synovial-derived MSCs to prevent cartilage degeneration in a rat partial meniscus defect model. We removed the anterior half of the medial meniscus and grafted autologous Achilles tendons with or without a 10-minute pretreatment of the tendon with synovial MSCs. The meniscus and surrounding cartilage were evaluated at 2, 4, and 8 weeks (n55). Tendon grafts increased meniscus size irrespective of synovial MSCs. Histological scores for regenerated menisci were better in the tendon 1 MSC group than in the other two groups at 4 and 8 weeks. Both macroscopic and histological scores for articular cartilage were significantly better in the tendon + MSC group at 8 weeks.

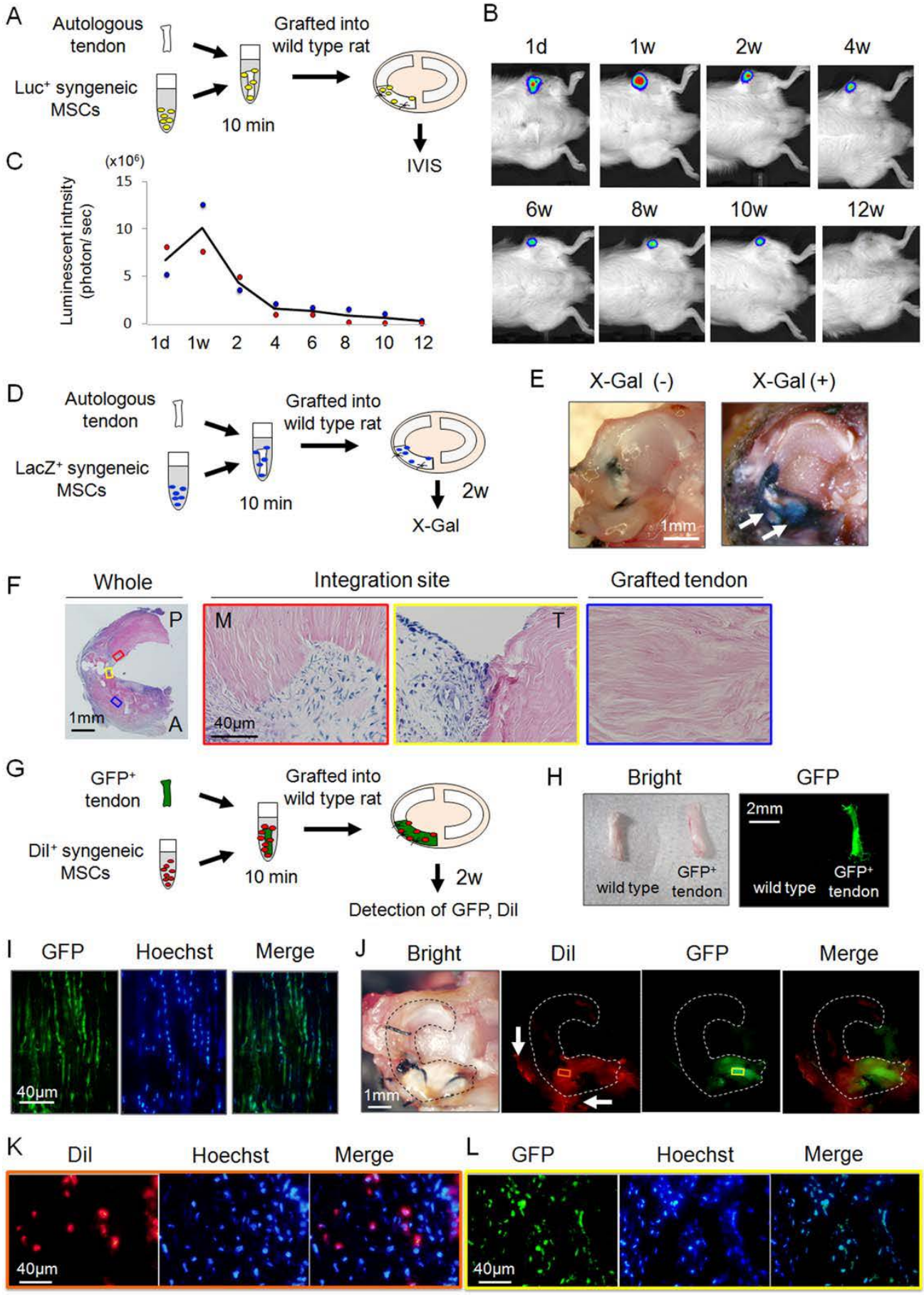
However, we have not been able to identify the relationship between transplanted MSCs, tendon grafts and surrounding cartilage tissues on the transplanted graft. The bioimaging rat developed by Prof. Kobayashi has played an important and indispensable role to scrutinize the correlation. The rat has been developed through the optimization of LEW (RT11) and cross-fertilization of 3 inbred strains as F1 Hybrid. The below imaging is achievable;



Implanted synovial MSCs survived around the grafted tendon and native meniscus integration site by cell tracking assays with luciferase, LacZ, DiI, and/or GFP synovial MSCs and/or GFP tendons. Flow cytometric analysis showed that transplanted synovial MSCs retained their MSC properties at 7 days and host synovial tissue also contained cells with MSC characteristics. Synovial MSCs promoted meniscus regeneration augmented by autologous Achilles tendon grafts and prevented cartilage degeneration in rats.

The below image Fig. 4 is picked up from the STEM CELLS 2015;33:1927–1938;

Figure 4. Detection of transplanted synovial mesenchymal stem cells (MSCs) and grafted tendon. (A): Schematic representation of the bioluminescent in vivo imaging analysis (IVIS). (B): Detection of photons from synovial MSCs derived from a luciferase expressing transgenic rat. (C): Sequential quantification of luminescence intensity. Raw data are plotted and the averaged values are shown as a line(n52). (D): Schematic representation of the detection of transplanted MSCs derived from a LacZ expressing transgenic rat (n52). (E): Macroscopic features after X-Gal staining for tibial plateau with medial meniscus. White arrows indicate the LacZ positive area. (F): Histological analysis of LacZ positive cells after X-Gal staining. The red and yellow squares show the integration site of native meniscus side and grafted tendon side respectively. The blue square shows the inner part of the grafted tendon. (G): Schematic representation of analyses of regenerated meniscus grafted with green fluorescence protein (GFP_1) Achilles tendon with DiI_1 MSCs (n52). (H): Macroscopic features of GFP_1 Achilles tendon. (I): Histology of GFP_1 tendon. (J): Macroscopic images of regenerated meniscus. The red or yellow squares show the site of histological analysis for DiI or GFP respectively. White arrows indicate the synovium which covered the grafted tendon. (K): Histological analysis of DiI_1 MSCs in the regenerated meniscus. (L): Histological analysis of GFP_1 tendon cells in the regenerated meniscus. Abbreviations: GFP, green fluorescence protein; IVIS, in vivo imaging analysis; MSC, mesenchymal stem cell.


A
Autologous tendon
Luc+ syngeneic MSCs
10 min
Grafted into wild type rat
IVIS
B
1d
1w
2w
4w
6w
8w
10w
12w
C
(x10^6)
Luminescent intnsity (photon/ sec)
15
10
5
0
1d 1w 2 4 6 8 10 12
D
Autologous tendon
LacZ+ syngeneic MSCs
10 min
Grafted into wild type rat
2w
X-Gal
E
X-Gal (-)
X-Gal (+)
1mm
F
Whole
Integration site
Grafted tendon
P
A
1mm
M
T
40μm
G
GFP+ tendon
DiI+ syngeneic MSCs
10 min
Grafted into wild type rat
2w
Detection of GFP, DiI
H
Bright
GFP
2mm
wild type
GFP+ tendon
wild type
GFP+ tendon
I
GFP
Hoechst
Merge
40μm
J
Bright
DiI
GFP
Merge
1mm
K
DiI
Hoechst
Merge
40μm
L
GFP
Hoechst
Merge
40μm