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幹細胞之訊息途徑及表基因調控--
子計畫六： 人類間質幹細胞供應中心

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目錄

Introduction to Core-B:

The Core B (hMSCs harvest Core) of this integrated study is conducted at the Stem Cell Laboratory of China Medical University Hospital. Based on the patient volume of CMUH and with the signed permit obtained from each patient, the Core is expected to collect 50 or more hBM-MSC annually to facilitate the experiments of project 1, 3, 4 and 5. In addition to providing MSCs for studies of other sub-projects, some experiments on MSCs were also conducted at Core B in this year and will be included in this report.

Research of BM-MSCs at Core B

Project 1: To study the effect of total body irradiation (TBI) on bone marrow-derived MSCs

Total body irradiation (TBI) is routinely used before allogeneic transplantation to kill the leukemic cells as well as to suppress the immunity of recipient to ensure donor hematopoietic stem cell engraftment. TBI is associated with a lot of long-term adverse effects on normal cells, including radiation-induced malignancies. In the series of Curtis *et al* studying 19229 recipients from allo- or syngeneic transplantation, the risk of solid tumors, including sarcoma – a cancer derived from mesenchymal tissue, was estimated to be 8.3 times higher than expected in the general population among 10 year survivors.

MSC is the stromal stem cell in the bone marrow and other mesenchymal tissue. Clinical application of MSCs is rapidly growing in recent years because of their multi-differentiation potential. The immunosuppressive property of MSCs can be applied to treat corticosteroid-refractory graft-versus-host disease in patients receiving allogeneic transplantation. Besides, the “tumor-homing” property of MSCs can be applied as cell therapy for cancer. However, the characteristics of MSCs isolated from patients who had received TBI have never been studied. Can MSCs isolated from TBI-exposed patients be safely used for patients of GVHD or TBI-associated solid tumors? Of more important, could high dose radiation of TBI transform the MSCs to become the origin of sarcoma in transplant survivors? We therefore conduct this study to elucidate the effect of TBI on bone marrow-derived MSCs.

Project 2: To study the "tumor-homing" specificity and the clinical effect of "tumor-homing" of bone marrow-derived MSCs in syngeneic animal model

Bone marrow-derived MSCs (BM-MSCs) can homing to injured tissue, including tumor tissue, and thus can potentially be used for cell therapy or as a vehicle for gene therapy. Most of prior studies were done on xenograft model and the homing of human BM-MSCs to human tumor tissue on immunodeficient mice had been criticized (human cells homing to human tissue rather than MSCs homing to tumor tissue). The condition of immunodeficient tumor model is also far away from the clinical condition of cancer. Besides, counting EGFP-expressed cell numbers in various tissues to examine MSCs homing specificity used in many prior studies may probably be subjected to significant bios between observers. A syngeneic model using in vivo image system (IVIS) is of great help to solve these problems.

In addition to the specificity of MSCs tumor-homing activity, the "clinical effect" of MSCs tumor-homing is also very important. There were conflicted data regarding to the effects of MSCs homing on tumor. Some experiments showed that MSCs homed to tumor and promote tumor growth. On the other hand, some experiments showed that MSCs homed to tumor can suppress tumor growth. This critical issue should be clarified clearly before trying to use MSCs as cell therapy or vehicle of gene therapy for cancer. We therefore conduct this study to set up this model to examine the BM-MSCs homing specificity and to see any effect (beneficial or detrimental effect) of MSCs tumor homing on "immunocompotent" animals.

Research Goals of Core-B

(1) Collect mesenchymal stem cells to support studies of other sub-projects
(2) Characterize the MSCs isolated from umbilical cord, bone marrow of normal adults and patients of haematological malignancies
(3) To study the effect of anti-cancer treatment (including radiotherapy, total body irradiation, chemotherapy, other drug therapies such as de-methylating agent Azacitidine or decitabine, proteasome inhibitor, thalidomide and lenalidomide…etc)
(4) To study the specificity and clinical effect of MSCs tumor-homing in syngenetic, immunocompotent animal model

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Methods

Collection and Characterization of MSCs

From Sep. 2008 to Sep. 2009, we had collected 21 specimens of bone marrow -derived MSCs from adult patients with hematological disease (table 1). The morphology of these cells is fibroblast-like. The phenotype, determined by flow cytometric analysis, is CD13+, CD29+, CD44+, CD73+, CD90+, CD105+, CD166+, CD14-, CD34-, CD45-, in most of them (Not shown here, please refer to annual report of last year). The potential of multi-differentiation into adipocyte, osteocyte, and chondrocyte is confirmed (Not shown here, please refer to annual report of last year).

Project 1 of Core-B: To study the effect of total body irradiation on the bone marrow- derived MSCs

Methods in Brief

- Ex vivo irradiate normal MSCs with 1200cGy (200cGy, twice daily, consecutive 3 days, mimic TBI in clinical practice) to see the effect of irradiation on surface antigen expression, proliferation, differentiation potential, immunosuppressive ability, and chromosomal integrity of normal MSCs.
- To confirm the data obtained from ex vivo irradiation experiments, we will harvest and ex vivo expanded MSCs isolated from leukemic patients before and after TBI (designated as BT-MSC and AT-MSCs respectively).
- Compare the surface antigen expression, proliferation ability, differentiation potential, immunosuppressive property, and chromosomal integrity of BT-MSCs and AT-MSCs.

Project 2: To study the "tumor-homing" specificity and the clinical effect of "tumor-homing" of bone marrow-derived MSCs in syngeneic animal model

Methods in brief

- Obtained D1 cell (BM-MSCs from balb/c mice) from ATCC
- Select firefly luciferase stably expressed D1 cells (D1-Luc)
- 4T1 (breast cancer cell line obtained from balb/c mice) cells inoculate subcutaneously onto balb/c mice
- After the establishment of breast tumor on balb/c mice, D1-Luc systemically

injected via tail vein to see the homing activity of D1-Luc to 4T1 tumor tissue using IVIS (xenogen).

- To test the specificity of D1 cell homing in other tumors, other cancer cell line from Balb/c mice including CT26 (colon cancer cell line) and Rag (renal cell carcinoma cell line) will also be done similar to 4T1 model.
- Our preliminary result showed D1-luc homing to 4T1 tumor specifically, we will further characterize the factor (such as tumor size) that may influence the homing activity of D1-luc.
- To examine the clinical effect of D1 homing to 4T1 tumor, we will compare the tumor growth and metastatic potential of balb/c mice bearing 4T1 tumor with or without systemic injection of D1 cells (i.e., 10 4T1-mice received D1 tail vein injection, 10 4T1-mice received PBS tail vein injection, then compare the tumor size, tumor metastasis, vascular density/tumor associated fibroblast within tumor in each group)

Results

Collection of MSCs and Connection with Other Sub-projects

(1) Already provided 6 bone marrow-derivede MSCs isolated from patients of multiple myeloma to Dr. Shih-Chie Hung (PI of Sub-project 1) to study the interaction between myeloma cancer cells and microenvironment cell (MSCs)

(2) Already provide 6 MSCs, 3 with normal differentiation potential, 3 with impaired differentiation potential, to Dr. Long-Yuan Li (PI of Sub-project 3) to study the signal pathway of adipocytic and osteocytic differentiation in the near future.

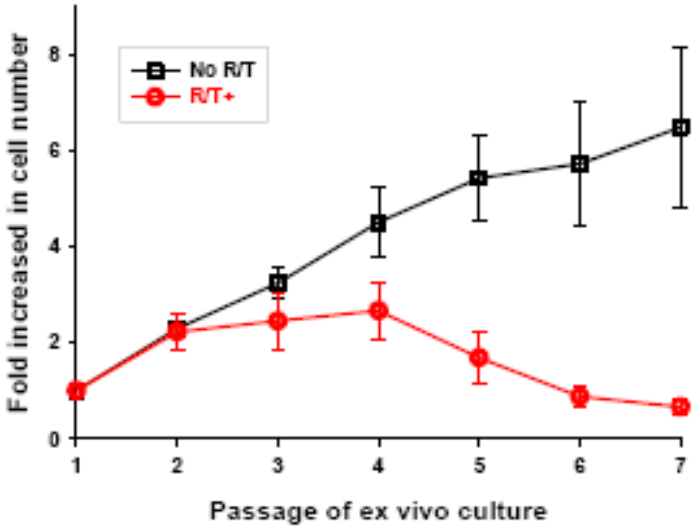
(3) Already provide 3 "pairs" of MSCs (3 before total body irradiation; 3 after total body irradiation) to Dr. Long-Yuan Li (PI of Sub-project 3) to investigate the molecular mechanism why the differentiation potential of MSCs is impaired after total body irradiation.

(4) Already provide 3 normal MSCs to Dr. Yung-Luen Yu (PI of Sub-project 4) to study the signal pathway of neuron differentiation.

(5) Already provide 2 normal MSCs to Dr. Mien-Chie Hung (PI of Sub-project 5) to study the role of EZH2 in stem cell differentiation and growth.

Result of Project 1 of Core-B: To study the effect of total body irradiation on the bone marrow- derived MSCs

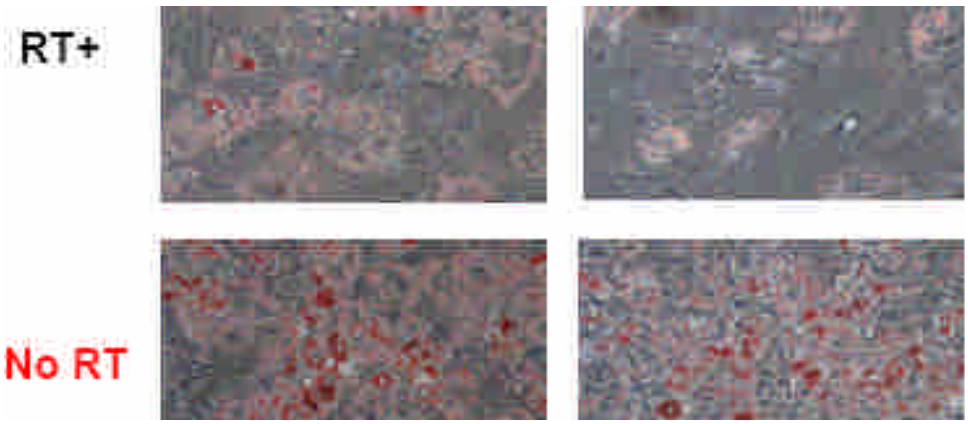
(1) Irradiation 1200cGy significantly dec. the growth of MSCs



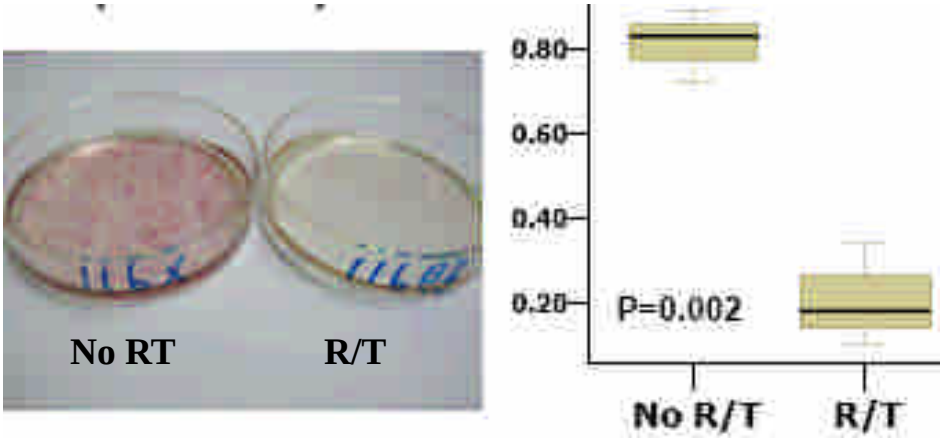
No R/T: 3 normal MSCs, subculture (passage) every 3 days

R/T +: 3 normal MSCs, irradiated with 1200cGy firstly, then sub-culture (passage) every 3 days

(2) Irradiation 1200cGy significantly dec. the differentiation potential of MSCs



Adipocytic differentiation, using Oil Red O stain



Osteocytic differentiation, using alkaline phosphatase stain

(3) Irradiation 1200cGy induced sustained cytogenetic abnormality

2 Normal MSCs irradiated with 1200cGy, then evaluate chromosomal integrity using whole chromosomal painting probes (Spectral karyotyping, SKY)

(4) MSCs isolated from BMT patients who had received TBI 1200cGy also have significant chromosomal abnormalities

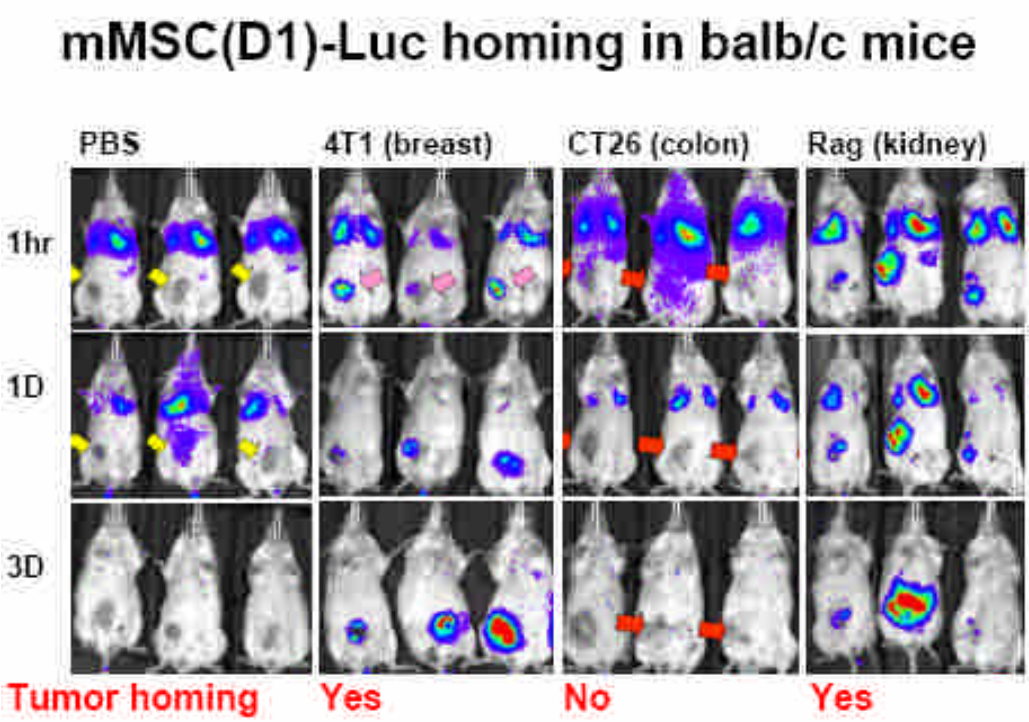
Cytogenetic Status of BM-MSCs Before / After TBI (Transplantation)					
No	Disease/ Gender	Conditioning Treatment	Chromosome of BM-MSCs		Chromosome of BM-blood cells after transplant
			Before TBI	After TBI	
1	AML/M	TBI/Cy	Not available	46 XY, t(7;22)(p22;q11), del (13)(q12q22), del (15)(a15?)	Normal XX
2	ALL/M	TBI/Cy	Not available	47 XY, del (1)(p36), +2, t(3;13)(p21;q12), t(5;10)(q33;p15)	Normal XX
3	AML/F	TBI/Cy	Normal XX	46 XX, inv (3)(p23;q29); t(14;15)(q24;q11)	Normal XY
4	AML/M	TBI/Cy	46 XY, del(1)(q42)	46 XY, del (1)(q42); del(5)(q13q22)	Normal XY
5	ALL/M	TBI/Cy	Robertsonian variant, 46 XY	46 XY, Robertsonian variant, t(1;17)(q21;p13)	Normal XX

Result of Project 2: To study the "tumor-homing" specificity and the clinical effect of "tumor-homing" of bone marrow-derived MSCs in syngeneic animal model

(1) Establish of firefly luciferase stably expressed D1 (BM-MSCs from Balb/c mice) cell line

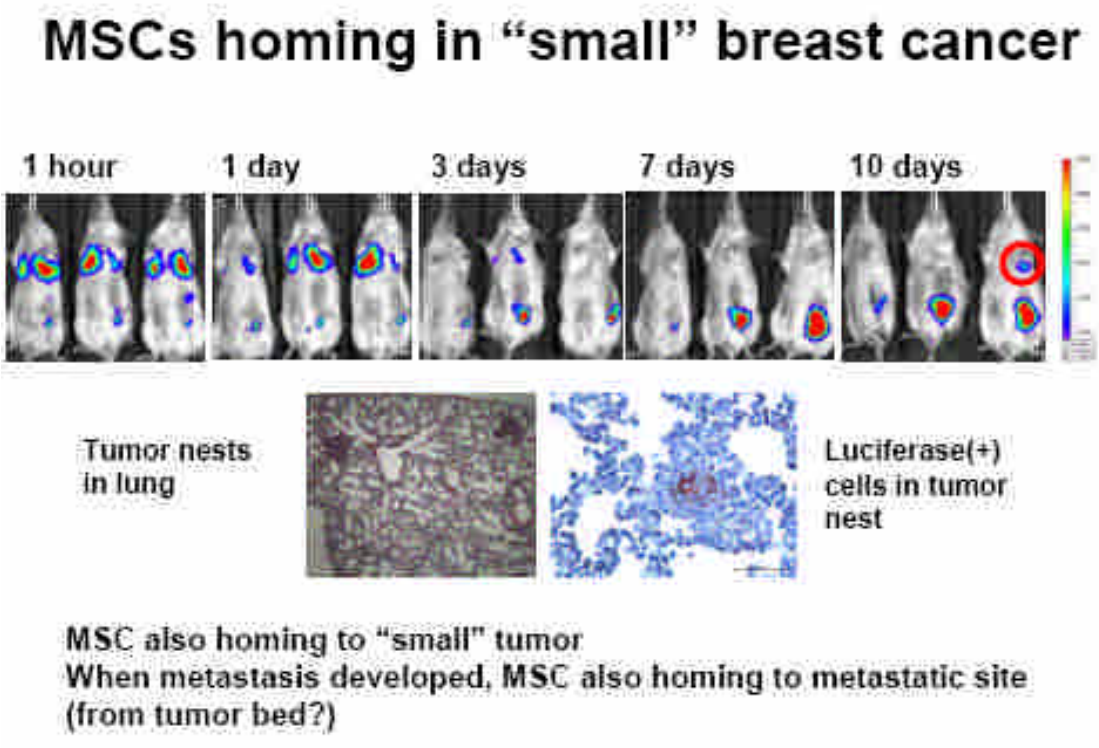
(2) D1-Luc can be easily traced in Balb/c mice (not shown here, please refer to annual report of last year)

(3) Compare D1-luc homing activity in Balb/c mice bearing 4T1 (breast cancer), CT26 (colon cancer), and Rag (renal cancer)

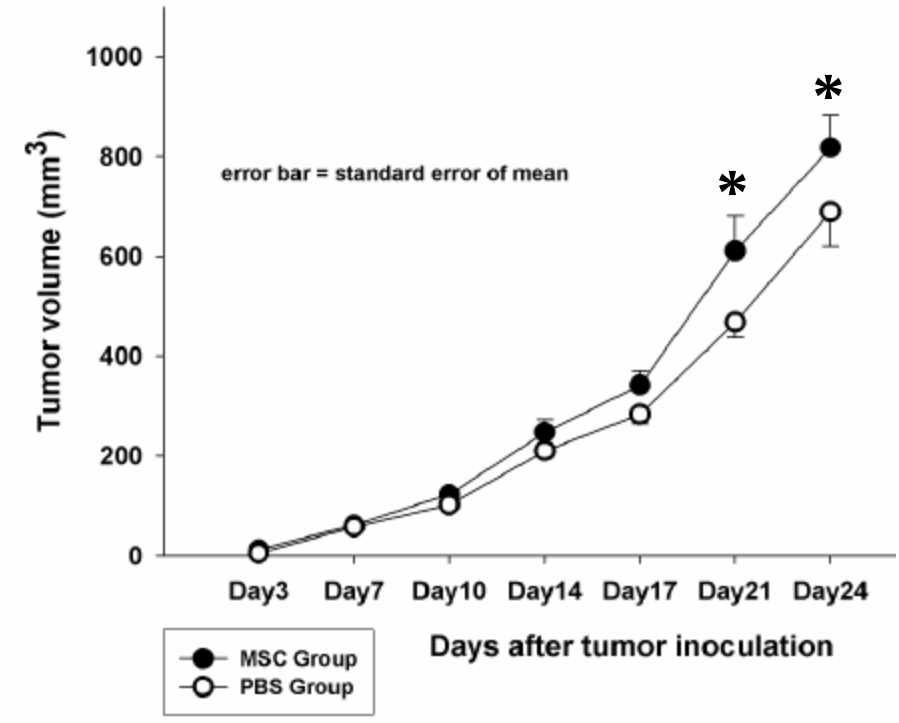


D1-Luc accumulated in lung 1 hour after tail vein injection. D1-Luc homing to 4T1 (big tumor, macroscopic tumor) and Rag but not CT26 tumor site 1 day and 3 days after tail vein injection.

(4) D1-Luc can also homing to small, impalpable 4T1 tumor sites



(5) D1-Luc homing to 4T1 tumor site and promote tumor growth and metastasis



D1-Luc significantly inc. tumor growth 21 days after tail vein injection

Bilateral lung of all 4T1-bearing mice were examined on Day 24 and developed all of them had pulmonary metastases. However, Mice with D1-luc injection has significant higher metastatic numbers than mice with PBS injection.

	D1-Luc group (total 8 mice)	PBS group (total 7 mice)	P value
Lung mets	8/8	7/7	
Metastatic sites in each lung	12.75 ± 5.39	7.57 ± 3.31	0.045 (t-test)
>= 10 metastatic sites in each lung	6/8	1/7	0.019 (Chi-square)

Discussion

1. Irradiation using total body irradiation dosing schedule (200cGy twice daily for consecutive 3 days) results in decreasing growth and differentiation potential of BM-MSCs. Besides, sustained chromosomal abnormality could be found on irradiated BM-MSCs. The same characteristics were further confirmed on BM-MSCs isolated from patients who had received total body irradiation.
2. Irradiated BM-MSCs have limited self-renewal, limited differentiation potential, and may have sustained chromosomal abnormalities. These cells should not be subjected for any clinical application. Besides, these cells may be the origin of post-irradiation soft tissue sarcoma.
3. The mechanism why irradiation reduces the adipocytic and osteocytic differentiation is now under investigation by Dr. Long-Yuan Lee of sub-project 3.
4. MSCs homing to tumor in syngeneic, immunocompotent animal model is well-established in this study. Not all tumors can attract MSCs homing to the tumor sites. In this study, breast cancer and renal cancer but not colon cancer can attract MSCs homing to tumor sites.
5. Using 4T1 (breast cancer) model, both big tumor and small tumor can attract MSCs homing to tumor. However, when comparing with control group (PBS injection via tail vein), MSCs tail vein-injected mice have higher tumor growth and higher potential of pulmonary metastasis. The clinical application of using MSCs for cancer cell therapy should therefore be very careful.
6. We are now look into the mechanism why MSCs homing to specific tumor type and how MSCs promote cancer cell growth and metastasis.

Publication:

1. Oral presentation at 2 international BMT symposium
2. Already submitted 2 abstracts to Annual Meeting of American Society of Hematology (ASH 2009).
3. The effect of irradiation on MSCs is prepared for submission.