Mesenchymal Stem Cells and Cancer: Their Interplay

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Cancers Are Major Healthcare Challenges

(Campbell-Walsh Urology, 9th ed, 2007, Saunder)
The disadvantages of traditional therapies

Problems of specific targeting?
Gene therapy methods are well established

• As of January 2007, there were a total of 1,260 gene therapy clinical trials approved worldwide, with approximately 90 new trials submitted for approval each year, most of which in the USA and Europe.

• Nearly half of all trials use one of two viral-based gene transfer vectors, adenovirus and retrovirus.

• Specific homing/targeting is still the challenge.
Mesenchymal Stem Cells
Bone Marrow Mesenchymal Stem Cells

- Easy to collect and expand
- Low immunogenicity
- Low tumorigenic/transformation rate
- Can be gene modified without affecting their phenotype
- Home to injury tissues and tumours/cancer
MSCs Can Home to Injury Tissues

MSCs home to a variety of tissues, particularly after tissue injury and ischemia.


Systemically Administrated MSCs Specially Home to the Tumours

Journal of the National Cancer Institute, Vol. 97, No. 7, 2005
Tumour development shares many common characteristics of wound healing; *Tumours are regarded as wounds that never heal.*

- Rapid cell proliferation and differentiation
- Angiogenesis
- Immuno-suppresive nature

Main Difference: *Tissue Remodeling: Apoptosis*
MSCs and Tumour Stroma

• Where do the tumour stroma originate?
  – Local
  – Circulating cells

• MSCs form tumour stroma.

• Tumor microenvironment select MSC engraftment.
The tumour environment recruits MSCs

Cytokines/Chemokines

Endocrine

Recruitment

Paracrine

Tumor mass
Carcinoma-associated fibroblasts (CAFs)

Tumor progression

Cancer cells and their cytokine receptors

Blood vessel

New cytokines/Chemokines production, further recruitment

Neutrophils
Mast cells
Cytokine/chemokine/growth factor

Lymphocytes

Stem/Progenitor cells

Macrophage/monocyte
Carcinoma Associated Fibroblasts, CAFs
MSCs-like cells increased in patient with chondrosarcoma, PBMNCs culture; 7 days; 40x
MSCs-like cells increased in patient with osteosarcoma, PBMNCs culture; 14 days; 40x

Flow cytometric analysis demonstrated an >9-fold increase in the number of cells with MSC-like phenotypes CD34(-)CD45(-)CD105(+) in patients with bone sarcomas compared with control subjects (p<0.05).

Bian, et al. Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas. Arch Med Res. 2009 Apr;40(3):163-8
BMSCs migration toward tumor cell lines before and after tumor condition medium stimulation

Numbers of cells migrated through transwell membranes

- Condition Medium Stimulation
- No Stimulation

A Sideview

B Topview

PC3  DU145  MCF-7  RIF-1

CM stimulate

No stimulate
BMSCs response to tumor cells in close contact
Wound healing assay of BMSCs in tumor cells condition media

DU145

PC3

CONTROL

Percentage of the Starting distance

12hrs 24hrs
The changes of CXCR4 and MMP-2 expression were confirmed by Western blot

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<tr>
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<th>PC 3</th>
<th>DU 145</th>
<th>RIF-1</th>
<th>MCF 7</th>
<th>Control</th>
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BMSCs migration towards to tumor cells before and after treating with CXCR4 and MMP2 inhibitor

Numbers of cells migrated through transwell membranes

- Tumor cell only
- AMD3100
- MMP2 Inhibitor
- Medium control

PC3, DU145, MCF7, RIF-1
MSCs as carrier for anti-tumour/cancer therapy

Significance

Systemic release

Cytokines

BM-MSCs

Tumours Cancers

Cell delivery

Systemic delivery

Genetic modification

Implications for clinical practice

Gene Therapy
IVIS ® 200 Imaging System: Tracing Stem Cells in vivo

- **In vitro**
  - GFP Rat
  - Luc-CMV Mouse
  - Luc-BMP-4 Mouse

- **In vivo**
  - bioluminescence
  - fluorescence

- 3D reconstruction
  - Co-registration with X-Ray
  - with PET & SPECT
  - with MRI & CT

- Chemiluminescence
Luciferase gene was stably transduced into BMSCs and tumour cell lines.
Good correlations were found between cell numbers and bioluminescence in all cells.
Study MSCs Homing to Tumours

TUMOUR CELLS SUBCUTANEOUS IMPLANTATION

Luciferase labelled MSCs

Tumour xenograft model

Luciferin injection
In-vivo imaging

TUMOUR CELLS SUBCUTANEOUS IMPLANTATION
MSCs distribution in PC3 tumor bearing mice

Day 3  Day 6  Day 9  Day 12
Study MSCs Homing to Tumours

TUMOUR CELLS SYSTEMIC INJECTION

Luciferase labelled tumour cells

Tumour metastasis model

Luciferin injection
In-vivo imaging
MSCs distribution in PC3 lung tumor model from day 1 to day 30. The cells mainly stay in lung where the tumor located. Proved by histology.
MSCs mainly home to tumours following systemic administration.
The engraftment of BMSCs in the tumour parenchyma was confirmed by immunostaining of GFP positive cells.
BMSCs did not favor tumour growth in short term co-culture

A. PC3 cells

B. DU145 cells

C. MCF-7 cells

D. RIF-1 cells
BMSCs favour PC3 tumour growth in vivo

E. Co-implantation of PC3 and BMSCs/Fibroblast

Tumour size mm$^3$

- MSC: PC3 1:1
- MSC:PC3 1:10
- Fibroblast:PC3 1:1
- Fibroblast:PC3 1:10
- PC3 only

6 days, 14 days, 19 days, 25 days
Herpes simplex virus 1 (HSV-1) TK enzyme is not expressed in normal cells.

HSV-TK converts GCV to triphosphate GCV (GCV-TP), which is cytotoxic.

GCV-TP causes cell death by apoptosis.
Stem cells for gene therapy

- Stem cells as gene delivery vehicles
- TK gene was transfected into MSCs using Lentiviral system

48h after C3H10T1/2 cells transfected with Lenti-CMV-luciferase-GFP virus.
The phenotype of Lenti-Luciferase-TK transduced BMSCs did not change
TK transduced BMSCs respond to GCV in 24 h

![Image of TK transduced BMSCs expression and GAPDH control.](image)

- Normal BMSCs
- Plasmids control
- TK BMSCs

TK expression
- 102bp
- 89bp

GAPDH

In presence of GCV

![Graph showing OD Value over time for different cell lines.](image)
Anti-tumour effect of systemically administered TK-MSCs in the presence of GCV in tumour bearing mice.
Systemic administration of TK-MSCs and GCV significantly inhibited metastatic tumour growth
Gross Sample of RIF-1 tumor in lung day 27

TK MSCs and GCV
TK MSCs only
GCV only
Percentage of BMSCs in the lung compared to total BMSCs in the body
Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma

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Keywords Fibrosarcoma, gene therapy, inducible nitric oxide synthase, mesenchymal stem cells.

Introduction

Solid tumors comprise two distinct but interdependent compartments: neoplastic cells and the stroma that the neoplastic cells induce and in which they are dispersed. Stem cells are mainly referred to as tumor-supporting fibroblasts and they may derive from resident fibroblasts in the organ/issue [1] or circulating mesenchymal progenitor cells [2-5].
Using Mesenchymal Stem Cells as Vehicles for Anti-cancer Therapy

Mesenchymal stem cells transduced with herpes simplex thymidine kinase
MSCs as anti-tumour gene therapy vehicle

- Transduction of the HSV-TK gene into BMSCs did not change their MSCs phenotype and tumour homing potential.

- Potent cytotoxic effects of TK-BMSCs/GCV was proved on tumour cells in vitro.

- TK-BMSCs together with GCV can greatly inhibit tumour growth in both local and metastasis tumour models in vivo.
Thank you!

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