Effect of Human Umbilical CordMesenchymal Stem Cells on Breast Carcinoma Therapy

Thesis

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Abstract

Background:

Breast cancer is the most common malignancy in women, accounting for 27% of all female cancers; it accounts for < 1% of all cancer cases in men. Breast cancer also is responsible for 15% of cancer deaths in women, making it the number-two cause of cancer death.

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs), isolated from discarded extra-embryonic tissue after birth, are promising candidate source of mesenchymal stem cells (MSCs). Apart from their advantages in abundant supply, painless collection, and faster self-renewal, hUC-MSCs have shown the potencies to differentiate into a variety of cells of three germ layer; hUC-MSCssynthesize and secret a set of trophic factors and cytokines to migrate toward inflammatory tissues such as cancer tissues

Methods:

This work was divided into two parts; in vitro work which involves human breast adenocarcinoma coculturewith human Wharton jellymesenchymal stem cells and in vivo work which involved transplantation of isolated hUC-MSCs into induced experimental mice with breast adenocarcinoma. We assessed cell proliferation by MTT assay, genes expression (TLR4, NF-KB, VEGF, IL-1, IL8) byqRT-PCRboth in vivo and in vitro, the level of IL-6 and JNK by ELISA technique from in vitro cultured conditioned media.

Results:

As regard cell proliferation assay there was significant decrease in breast carcinoma groups compared to all other treated cancer groups.As regard genes expression there was significant decrease in (TLR4, NF-KB, VEGF, IL1and IL-8) genes and significant decrease in (IL-6, JNK) level which are responsible for development and progression of cancer, in breast carcinoma groups compared to all other treated cancer groups.Better results were obtained by addition of doxorubicin toMSCSin treatment of breast carcinoma.

Conclusion:

HUC-MSCS can be applied in breast carcinoma treatment, as it affects the mechanism of tumor regression through modulation of different genes and cytokines expression which are involved in tumor initiation and progression. But much better results were obtained on combination between MSCS and doxorubicin for breast cancer treatment.

Key words: breast carcinoma, Wharton jellyhMSCs,doxorubicin.

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List of abbreviation

AC	Adriamycin
AP	alkaline phosphatase
AS	antisense
ATCC	American Type Culture Collection
ATM	The ataxia-telangiectasia mutated
AT-MSC	Adipose- derived stem cells
BM-MSC	Bone Marrow Mesenchymal Stem Cell
BR	Breast cancer
CAT	cyclophosphamide, Ara-C and topotecan
CB	Cord blood
CD	*clusters of differentiation*
cDNA	Complementary DNA
CHEK2	Checkpoint kinase 2
CMF	Cyclophosphamide · Methotrexate · Fluorouracil
CPG	cytidine phosphate guanosine
CRC	Colorectal cancer
CRP	c-reactive protein
CT	Computed tomography
$DAMP_S$	Danger-associated molecular pattern
DCIS	Ductal carcinoma in situ
DEPC	Diethylpyrocarbonate · 1,2-Erucoyl-sn-Glycero-3-phosphocholine
DES	Diethylstilbestrol
DKK-1	dickkopf-1
DLI	Donor lymphocytic infusion
DMEM	Dulbecco's modified Eagle's medium

DNA	Deoxy ribonucleic acid
dNTPs	Deoxynucleotide triphosphate
ECFC.	*endothelial colony-forming cells*
ELISA	Enzyme linked immune sorbent assay
EPC	Endothelial progenitor cell
ER	Estrogen receptor
ERK	extracellular signal-regulated kinase
FACS	Fluorescence-activated cell sorting
FBS	fetal bovine serum
G-CSF	*granulocyte colony-stimulating factor*
GTC	guanidine thiocyanate
GVHD	Graft versus host disease
GVT	Graft versus tumor
H,E	Hematoxlin and eosin
HDC	High dose chemotherapy
HE	hematoxylin and eosin
HER2	human epidermal growth factor receptor 2).
HLA	Human leukocytic antigen
HPRI	Human Placental Ribonuclease Inhibitor
HRP	*horseradish peroxidase*
HSC_S	Hematopoietic stem cells
HSCT	Hematopoitic stem cell transplantation
$hWJSC_S$	Human Wharton jelly stem cells
IgG	Immune globulin G
IKK	I-kappa B kinase complex
IL	Interlaken
INFs	interferons
IRAK	interleukin-1 receptor-associated kinase

IRFs	Interferon regulatory factors
IRFs	interferon regulatory factors
JNKs	c-Jun N-terminal kinases
KS	Kaposi sarcoma
LC	Lung cancer
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
LPS	Lipopolysaccharide
LRRs	Leucine-rich repeats
Mal	MyD88 adaptor-like protein
MAPKs	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-i
MHC	Major histocompatibility
MKP	mitogen-activated protein kinase phosphatase-1
MMLV	Moloney murine leukemia virus
MNC	Mononuclear cell
MRI	Magnetic resonance image
MSC_S	Mesenchymal stem cells
MTOR	Mammalian target of rapamycin.
MTT	4,5-dimethylthiazol-2yl]-2,5-diphenyl-tetrazolium bromide
MYD88	myeloid differentiation factor 88
NEMO	NF-kappa-B essential modulator
NF-KB	Nuclear factor kappa B
NF-KP	Nuclear factor kappa -light-chain-enhancer of activated B cells
NK	Natural killer cells
NOS	Not otherwise specified

OC	Ovarian cancer
$PAMP_S$	Pathogen associated microbial
PB-MSC	Peripheral blood mesenchymal stem cells
PBS	phosphate buffer saline
PBST	Phosphate Buffered Saline (PBS) solution with the detergent Tween
Pdcd4	Programmed cell death 4
PET	positron emission tomography
PGN	Peptidoglycan
PI3K	Phosphoinositide 3-kinase
PI3K/AKT	Phosphoinositide-3-kinas
$PL\text{-}MSC_S$	Placenta derived mesenchymal stem cell
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
RAS/MEK/ERK	Mitogen-activated protein kinase –ERK kinase
RCC	Renal cell carcinoma
RFUs	relative fluorescence units
RICT	Reduced intensity conditioning regimens
RNA	Ribo nucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SAPK	Stress-activated protein kinase
SCLC	Small cell lung carcinoma
SCT	Stem cell transplantation
TAK1	Transforming growth factor ß activated kinase-1
Taq	TaqMan
TICAM2	TIR domain-containing adapter molecule 2
TIRAP	TIR-associated protein
TLR	Toll like receptor

TLR	Toll like receptor
TNF-α	Tumor necrosis factor
TRAF	TNF receptor associated factor
TRAM	TRIF-related adaptor molecule
TRIF	TIR domain-containing adaptor protein-inducing IFN-β
UC	Umbilical cord
UCMSC	Umbilical cord mesenchymal stem cell
VACCERA	The Holding Company for Biological Products and Vaccines
VEGF	Vascular endothelium growth factor

Introduction & Aim of the work

Breast cancer (malignant breast neoplasm) is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts are known as ductal carcinomas; those originating from lobules are known as lobular carcinomas. Breast cancer is a disease of humans and other mammals.

Human umbilical cord (UC) has been recently suggested as a valid alternative tissue for MSCs. The UC is a tissue of extraembryonic origin lying between the mother and the fetus, consisting of two arteries, one vein, intervessels connective tissue (the Wharton's jelly), and umbilical epithelium.

The UC is normally discarded after birth. Therefore, UC collection does not require any invasive procedure nor implies major ethical concerns. MSCs have been isolated from all compartments of the umbilical cord tissue, namely, the umbilical vein endothelium and subendothelium and the Wharton's jelly. Within Wharton's jelly, MSCs have been isolated from three regions: the perivascular zone (UC perivascular cells), the intervascular zone, and the subamnion.

Fong et al., 2012, reported that BM-MSCs have been used for various cell-based therapies but have the limitations of painful harvest, morbidity, and risk of infection to the patient. This prompted researchers to explore the use of human umbilical cord Wharton's jelly MSCs (hWJSCs) and its conditioned medium (hWJSC-CM) because hWJSCs can be harvested in abundance painlessly, are proliferative, hypoimmunogenic, and secrete a variety of unique proteins. They proposed that cord blood banks freeze autologous hWJSCs and

umbilical cord blood (UCB) from the same umbilical cord at the same time for the patient for future cell-based therapies.

Wharton's jelly-derived MSCs have shown multilineage capability along with immune regulatory properties. It has been shown that a single injection of MHC mismatched unactivated human UC-MSCs did not induce a detectable immune response; therefore, they can be tolerated in allogeneic transplantation.

Advantage of UCMSCs

- Widespread availability.
- Absence of donor risk.
- Low risk of transmissible infectious diseases.
- Decreased graft-versus-host disease.
- Increased precursors of immune effector cells.

Toll-like receptors, a mammalian homologue of the drosophila toll protein, are the best-characterized family of pattern-recognition receptors (PRRs), which sense foreign material, so called pathogen-associated molecular patterns (PAMPs), derived from bacteria or virus. After activation of TLRs with their ligands either directly or with help of accessory proteins such as CD14 and MD2 (in case of TLR2/4), cells evoke inflammatory response, coordinate the immune system in the whole organism, and finally protect the host from spreading massive pathogen after infection.

It has been known that TLR expression in many tumors or cell lines was up-regulated. Also, in some tumor models, polymorphisms of TLR2 and 4have been known to influence the risk of cancer, implicating that genetic variation in specific TLR may be associated with specific tumor progression. TLR4 has been known as indicative molecule for detection of predisposition to a cancer.