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Anti-tumor Potentials of various Mesenchymal Stem Cell types using two known Cancer Cell Lines

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Abstract: **The discussions, debates and research on Pro-cancer and anti-cancer nature of MSCs have not yielded any clear conclusion yet. MSCs in this regards are specific, based on various factors like source of derivation, the specific cancer cells under study, *in vitro* or *in vivo* conditions provided in the study, to name a few. TRAIL (Tumor necrosis factor related apoptosis inducing ligand) reportedly induces apoptosis in cancer cells; however, CXCL12/CXCR4 axis overrides most of the apoptotic signals and leaves TRAIL ineffective. These studies were performed on MSCs derived from the bone marrow and cord tissue. Perinatal tissue derived MSCs and MSCs induced by different factors have not been researched for this behavior.**

Objective: To study anti-cancer nature of MSCs derived from sources such as perinatal tissues (Cord tissue, placenta and amniotic sac) and adipose tissues on two chosen tumor cell lines namely small cell lung carcinoma (A549) and prostate carcinoma (PC-3) cells. The use of modified MSCs, by inducing MSCs with TNF-α and LPS (Lipopolysaccharide) was the next objective. In this study, we focused on regulatory pattern of TRAIL and CXCL12/CXCR4 pathway genes, the various expression patterns of tumor suppressor genes, Tumor Activating Fibroblasts and the Wnt pathway genes.

Results: MSCs induced with TNF-α demonstrated better anti-cancer characteristics on A549 lung Carcinoma cells. There was practically no effect on PC-3 cells by any of the MSC types as evaluated through apoptosis and cell cycle assays. The results were backed by observations on the regulatory patterns of various genes.

Conclusion: TNF-α induced MSCs impose anti-cancer properties on A549 cells through a specific TRAIL mediated apoptotic activity. The detailed mechanisms of action remain to be elucidated. PC-3 cells did not respond to anti-cancer cues using any of the MSC types. The Anti-cancer potential of MSCs is not likely to be uniform across cancers, and largely depend on the origin of MSCs and various other factors.

Keywords: **anti-cancer nature, Anti-cancer potential, Carcinoma cells, Tumor Activating Fibroblasts.**

I. BACKGROUND

Tumor Necrosis Factor-related Apoptosis Inducing Ligand (TRAIL) engineered into Mesenchymal Stem Cells (MSCs) exerts anti-tumor effect on solid tumors. This is a widely tested strategy of exploiting tumor–tropic properties of MSCs. [1] [2]. Many other anti-tumor factors were engineered into MSCs and tested for their effect if any, on solid tumors [2]. However, the stability of such genetically engineered MSCs is under scrutiny and is a challenge faced by researchers

The conventional therapeutic modalities like radiotherapy, chemotherapy, surgical resection and monoclonal antibody administration have their own limitations. They don't address tumor recurrence and metastasis after the initial induction of remission. These conventional therapies are devoid of precise specificity to target only the cancer cells and hence pose damage to the normal cells as well. The relapse or tumor recurrence after initial induction of remission is probably due to presence of cancer initiating cells or cancer cell stem cells (CSCs) [3][4].

Most of the studies conducted to test anti-cancer properties of MSCs were either genetically engineered or were isolated from bone marrow and adipose tissues. This study is an attempt to understand if MSCs derived from perinatal tissues possess any inherent anti-cancer properties., In this paper, we have tried to bring out these behavioral pattern using various MSC types' towards only two chosen cancer cell lines namely A549 (Small Cell Lung Carcinoma Cells) and PC-3 (Prostate Carcinoma Cells).

II. INTRODUCTION

Mesenchymal Stem Cells (MSCs) are master stem cells that are believed to be the 'cells' that will change the phase of Regenerative Medicine in the future. They possess multiple unique properties namely immune priviledgeness [2], ability to migrate to the site of injury or tumor, [5] and multilineage differentiation capabilities into all three germ layers [2]. The ease of availability is yet another virtue of stem cells. MSCs can be isolated from bone marrow, perinatal tissues, adipose tissue and from virtually all tissues of the human anatomy [6] [7]. The tumor tropism property of MSCs has been exploited by many researchers to explore delivery of anti-cancer agents to the site of tumor in various cancers [2] [6] [8] [9] [10] [11] [12] [13].

MSCs have shown to exert pro-tumor and anti-tumor effects on certain cancer cell' types which are dependent on various factors; these include but limited to the source of stem cells, the type and stage of cancer, the age of the patient, and the nature of priming of stem cells to up-regulate anti-cancer factors [2]. In some studies, the pro-tumorigenic property of MSCs has been attributed to secretion of pro-tumorigenic factors, promotion of vasculature and establishment of conducive microenvironment for cancer stem cells [14] [15] [16] [17] [17]. In our own review paper (Ramdasi et al 2015), we discussed and analyzed in details the various factors probably affecting anti-cancer nature of MSCs. These anti-cancer properties of MSCs have been tested successfully in the models of Pancreatic cancer [19], Lewis lung cancer [20], prostate carcinoma [21], glioma [22], breast cancer [23], colon cancer [24] and malignant melanoma [25].

The priming of MSCs is a very useful strategy to up-regulate secretion of inherent anti-cancer agents. In one study, researchers primed MSCs derived from the bone marrow and cultured cells to obtain subtypes MSC1 and MSC2 [26]. In this study MSC1 exhibited a 'pro-inflammatory nature' while MSC2 exhibited an 'immunosuppressive' nature. The follow up study by the same group demonstrated that the MSC1 are anti-cancer in behavior while MSC2 are pro-cancer in nature [27]. Most of the studies in this domain have been carried out using MSCs derived from the bone marrow and occasionally using adipose derived MSCs. Thus, in this study, we wanted to check if naive MSCs and primed MSCs derived from perinatal tissues possess any such anti-cancer properties at all!!

So, we explored the behavior of mixed MSCs, as shown in the subsequent text, derived from perinatal tissues (umbilical cord tissue, placenta and amniotic sac) towards two well characterized cancer cell lines viz. A549 (Small cell lung carcinoma) and PC-3 (Prostate carcinoma). Also, we conditioned 'Mixed MSCs' using recommended chemical agents for priming and up-regulating TRAIL (Tumor necrosis factor-Related Apoptosis Inducing Ligand) and evaluated their effect on tumor.

III. MATERIALS AND METHODS

A. Cancer Cells & Cell Culture

❖ ***Cancer Cells under Study***

We chose to use only two well established cancer cell lines for our experiments. Small cell Lung carcinoma cell line A549 (ATCC® CCL185TM) & Prostate carcinoma PC-3 cells (ATCC® CRL1435™)

❖ ***Culture and Expansion of Small Cell Lung Carcinoma (A549) cell line & Prostate Carcinoma (PC-3) cell line***

These Cell lines were cultured and expanded as per the manufacturer's instructions. Briefly, cells were cultured only whenever required, using cancer cell expansion media containing DMEM/F12 (Hi Media, India) with 10% Fetal Bovine Serum (Hi Media, India) supplemented with Penicillin-Streptomycin (Gibco, USA). Cells were harvested at >90% confluence.

B. Determination of Doubling Time of Cancer Cells

Doubling time of both the cancer cell lines, A549 and PC-3 was determined by performing growth curve assays.

Briefly, each well of six well plate (Hi Media, India) was seeded with 0.1 million cancer cells in cancer cell expansion media. The plates were then incubated at 37°C with 5% CO_2. After every 24 hours, one well was harvested, and cells were counted by Coulter counter, occasionally by manual method using hemocytometer. The experiment was carried out in triplicate, and results were reported as a Mean ± SD.

C. Isolation and Expansion of MSCs from Human Perinatal Tissues

MSCs from the three human perinatal tissues namely placenta, amniotic sac and umbilical cord tissue were isolated and expanded following a previously well standardized protocol [5] [7].

❖ ***MSC Isolation and Culture from Placenta, Amniotic Sac & Umbilical Cord Tissue***

Perinatal tissues from full term normal deliveries were collected after obtaining an informed consent from donor mothers. The informed consent and the isolation protocol was approved by the Institutional ethics committee.

The cells were allowed to expand in a monolayer culture at 37^0C and 5% CO_2. The media was changed every second day and cells were sub-cultured upon confluence and characterized appropriately.

D. Isolation and Expansion of Mixed MSCs - Mixing and Co-Culture of MSCs from Three Perinatal Sources

A mixture of MSCs from the three perinatal sources were co-cultured to check if they had any added advantage over MSCs isolated from an individual tissue. Briefly, the MSCs at passage zero (after primary culture) from all the three sources (cord tissue, placental tissue and amniotic sac) were co-cultured into a tissue culture-treated flask at a ratio of 1:1:1 in a similar expansion media as described previously [5] [7]. The media was changed every alternate day and cells were passaged upon confluence and characterized appropriately.

E. Isolation and Expansion of MSCs from Adipose Tissue

Approximately 50 ml lipoaspirate was collected after obtaining informed consent from donors. The in-house validated protocol was applied as reported previously [5] [7]. Briefly**,** lipoaspirate was washed several times to remove excess oil and then subjected to collagenase type VIII digestion. The cells were then expanded in MSC expansion media. Cells were incubated at 37°C with 5% CO_2. The media was changed every alternate day; cells were passaged upon 80-90% confluence. Cells were characterized appropriately.

F. Priming of Mixed MSCs with LPS and TNF-α

Mixed Mesenchymal stem cells were induced with LPS (Lipopolysaccharide) and TNF-α (Tumor Necrosis Factor Alpha) as described previously by Waterman et al [26] [28]. Mixed Mesenchymal stem cells (Mixed MSCs) were seeded into 75 cm^2 tissue culture flasks (Nunc, China) with MSC expansion media and incubated at 37°C with 5% CO_2 for 48 hours. After 48 hours the expansion media was replaced either with TNF-α induction media [MSC expansion media + 100 ng/ml TNF-α (Hi Media, India)] and/or LPS induction media [MSC expansion media + 100 ng/ml LPS (Sigma-Aldrich Inc., USA)] and incubated for another 48 hours at 37°C with 5% CO_2 for induction of MSCs. After 48 hours of induction, the induction media was replaced with MSC expansion media and cells were grown up to 80-90% confluence. Cells were then harvested using Trypsin (Trypsol-AOF®, Biogenomics, India) and characterized using markers CD105, CD90, CD73, CD45 and IDO (Indoleamine 2,3-dioxygenase) on flow cytometer. 10,000 events were acquired using FACSCalibur (BD Biosciences, San Jose, California) and analyzed using CellQuest Pro software provided by BD Biosciences.

G. Characterization of MSCs

❖ ***Morphology by Microscopy***

MSCs were observed everyday under the inverted light microscope (Nikon). Images of morphology were captured and recorded. Cells were observed for attachment to the plastic surface.

❖ ***Establishing Identity by Flow Cytometry***

The identity of MSCs was established by Flow cytometric analysis following ISCT (International Society for Cellular Therapy) guidelines [29] for MSC cell surface markers. Briefly, MSCs were harvested upon 80-90% confluence. Approximately 1 million cells were stained with fluorochrome tagged antibodies; CD45 PerCp (BD Biosciences, USA), CD90 FITC (BD Pharmingen, USA), CD73 PE (BD Pharmingen, USA), CD105 PE (BD Pharmingen, USA), IDO (Thermo Fisher, USA), 7-AAD (BD Biosciences, USA). Isotype controls were used for FITC, PerCp and PE antibodies. Rb X ShIgG FLUOR (Merk) antibody was used as secondary antibody to IDO (Indoleamine 2, 3-dioxygenase). Approximately 10,000 events were acquired on flow cytometer and analyzed appropriately. Only the MSC mixture harvested were subjected to flow for baseline characteristics. Individual MSCs' flow were performed otherwise at numerable occasions and their pattern, are well known and established already.

Cryopreservation and Storage of MSCs & Cancer Cells

All types of MSCs and cancer cells were cryopreserved using freezing media containing DMEM/F12 (Hi Media, India) with 20% Fetal Bovine Serum (Hi Media, India) and 10% DMSO (Sigma-Aldrich Company, USA). Cells were control rate frozen at -80°C in Mr. Frosty (Nalgene) freezing container overnight and transferred to liquid nitrogen vessel storage container (MVE-2000) on the next day. These cryopreserved cells were used as and when required.

H. Co-culture Assay (Cancer cells Vs. MSCs)

To investigate whether or not MSCs exert anti-cancer effect on cancer cells in a co-culture system, a co-culture assay was performed using cell culture inserts in six well plates (0.4µ pore size; Polyester; Hi Media, India) and six well plates (Hi Media, India). The inserts and their respective bottom wells were pre-incubated with 1.5 ml and 2.5 ml MSC expansion media respectively as per manufacturer's instructions and were incubated at 37°C for not less than one hour for initial equilibrium.

Mixed MSC (Mixture of MSCs from perinatal sources), TNF-MSC1 (MSCs induced by TNF-α), LPS-MSC1 (MSCs induced by Lipopolysaccharide) and ATMSC (Adipose Tissue derived MSCs) were thawed and cultured in MSC expansion media containing in 75 cm^2 tissue culture flasks at a density of 0.5 million cells each flask followed by incubation at 37°C with 5% CO_2. Upon reaching 80-90% confluence MSCs were harvested using Trypsin (Trypsol-AOF®, Biogenomics, India).

Small cell lung carcinoma cell line A549 (ATCC® CCL185™) and prostate carcinoma cell line PC-3 (ATCC® CRL1435™) were thawed and cultured in cancer cell expansion media as described previously. Upon reaching 80-90% confluence, cancer cells were harvested using Trypsin (Trypsol-AOF®, Biogenomics, India). Cells were counted as earlier described.

Human Foreskin Fibroblast (HFF) cells were used as negative control. HFF cells were thawed and cultured in cancer cell expansion media in 75 cm^2 tissue culture flasks at a density of 0.5 million cells per flask followed by incubation at 37°C with 5% CO_2. Upon reaching 80-90% confluence, HFF were harvested using Trypsin (Trypsol-AOF®, Biogenomics, India) and counted.

For setting up of the assay, we used cell culture inserts of a standard pore size of 0.4µ made up of Polyester, purchased from Hi Media, India. Each well of the six well plates (Hi Media, India) was seeded with 0.30 x 10^6 numbers of A549 cells in one set and PC-3 cells in another set of experiment in 2.5 ml/well of MSC expansion media. Refer to **Table number 1** for assay set up details.

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Table 1) Cancer Cell Vs. MSC Co-culture Assay Set up: Co-culture assay of cancer cells and MSCs using Cell culture inserts for six well plates.

Sr. No.	Cancer Cell Type 0.30 X 10^6/Well (Lower Well of 6-well Plate)	MSC Type 0.30 X 10^6/Insert (Upper Cell Culture Insert)
Assay Set up for A549 Vs. MSCs (Triplicate)		
1	A549	Mixed MSC
2	A549	ATMSC
3	A549	TNF-α MSC1
4	A549	LPS MSC1
5	A549	HFF
6	A549	Media Control – No MSCs
Assay Set up for PC-3 Vs. MSCs (Triplicate)		
7	PC-3	Mixed MSC
8	PC-3	ATMSC
9	PC-3	TNF-α MSC1
10	PC-3	LPS MSC1
11	PC-3	HFF
12	PC-3	Media Control – No MSCs

Six well plates with cancer cells alone were then incubated at 37°C with 5% CO_2 for 2 hours to allow attachment of cells to the bottom of wells. After 2 hours, the cell culture inserts were placed on each well and respective MSC type (**Refer Table 1**) were seeded on to the insert at a density of 0.30 x 10^6 MSCs/insert in 1.5 ml MSC expansion media. One insert was seeded with HFF as negative control and one insert was not seeded with any MSC (Media control). Six well plates then incubated at 37°C with 5% CO_2 for 48 hours. After 48 hours, cancer cells from each well and MSCs/HFF from all inserts were harvested using Trypsin (Trypsol-AOF®, Biogenomics, India). Cells were counted appropriately.

I. Apoptosis Analysis (Annexin V FITC Apoptosis Detection)

Apoptotic activity of cancer cells obtained from co-culture experiments was measured using Annexin V FITC Apoptosis Detection Kit II (BD Pharmingen, San Diego, CA) following manufacturer's instructions. Briefly, cells were washed twice with l x ice-cold PBS and resuspended in lx binding buffer (provided with the kit) at a concentration of 1 X 10^6 cells/ml. l X 10^5 cells (100 µl cell suspension) were then transferred to Polystyrene round bottom tubes for flow cytometry (5 ml; BD Falcon, Bedford, MA). 5 µl of Annexin V conjugated with Fluorescein isothiocyanate (FITC) and 5 µl of Propidium Iodide (PI) were added, then gently vortexed and incubated in dark at room temperature for 15 minutes. To each tube 400 µl of 1x binding buffer was added and approximately 10,000 events were acquired for flow cytometry.

J. Cell Cycle Analysis

The cell cycle was performed by DNA staining method using PI (Propidium Iodide; Hi Media, India). Briefly, after harvesting of cancer cells, 2 X 10^6 cells were pelleted at 1300 rpm for 5 minutes at 4°C. The pellet was washed with lx ice-cold PBS and again pelleted out at 1300 rpm for 5 minutes at 4°C. It was then resuspended in 500 µl ice-cold PBS and to this cell suspension, 500 µl 70% (v/v) ice-cold Ethyl alcohol (Hi Media, India) was added gently drop-wise on vortex to avoid clumping. The pellet was then stored on ice for 1 hour in dark. After fixation, cells were pelleted out, washed with ice-cold lx PBS. Finally, the pellet was resuspended in 500 µl ice-cold PBS and transferred to Polystyrene round bottom tubes for flow cytometry. The RNA contamination was removed by adding 12.5 µl RNase A (1 mg/ml; Thermo Fisher) and incubated at 37°C for 1 hour. 25 µl of Propidium Iodide (PI; 1 mg/ml; Hi Media, India) was added and incubated in the dark at 4^0C. The tubes were carried on ice to the flow cytometer. 10,000 events were acquired on 'slow' mode using flow cytometer to achieve best resolution and analyzed appropriately.

K. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

We studied the expression of various Tumor Activating Factors, Tumor Suppresser Genes, genes involved in Wnt signaling and CXCL12/CXCR4 pathway. We also looked for inhibitors of Wnt pathway in cancer cells and in MSCs after MSCs' co-culture using semi-quantitative RT-PCR.

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The cell pellets obtained from cancer cells (A549 and PC-3) and MSCs (Mixed MSC, TNF- MSC1, LPS-MSC1 and ATMSC) were subjected to total RNA extraction using RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. The RNA concentration was determined using NanoDrop UV-Visible spectrophotometer (Nanodrop Instruments). One microgram of RNA was converted to complementary DNA (cDNA) utilizing Superscript III First Strand Synthesis System (Thermo Fisher, USA) following the manufacturer's instructions. cDNAs from cancer cells and MSCs were amplified using the primers as shown in **Table 2**. The band intensity was qualitatively assessed by visual inspection.

IV. STATISTICAL ANALYSIS

The data was analyzed using 'student's unpaired 2 tailed t-test' using an online calculator provided by www.graphpad.com to determine the statistical significance. Values are given as mean ± SD (standard deviation).

Table 2) List of Genes and respective Primers Assessed by Semi-Quantitative Reverse Transcription PCR for cDNA obtained from Cancer Cells and MSCs

Gene	Forward / Reverse	Annealing Temperature (^{0}C)	Product Size (bp)
Genes Assessed in Cancer Cells (A549 and PC-3)			
β-Catenine	AAAATGGCAGTGCGTTTAG	50.3	100
	TTTGAAGGCAGTCTGTCGTA		
CXCR4	CTCTCCAAAGGAAAGCGAGGTGGACAT	65.6	558
	AGACTGTACACTGTAGGTGCTGAAATCA		
CXCR7	TAAATATATGCCAGTCTTGGCTGA	59.7	103
	TTACAAAGCAGTTTTCGTTCCATA		
C-MYC	TACCCTCTCAACGACAGCAG	59.7	478
	TCTTGACATTCTCCTCGGTA		
APC	CGCTCAACTTCAGAATCTCA	59.6	575
	CAGACTGTCGCATGGATATAC		
CYCLIN D1	CACCTAGCAAGCTGCCGAACC	59.8	219
	CGACAGACAAAGCGTCCCTC		
DKK1	ATTCCAACGCTATCAAGAACC	59.5	384
	CCAAGGTGCTATGATCATTACC		
BAX	TGCTTCAGGGTTTCATCCAG	59.5	170
	GGCGGCAATCATCCTCTG		
BCL2	GGCTGGGATGCCTTTGTG	59.5	66
	CAGCCAGGAGAAATCAAACAGA		
BIRC5	TGCTTCAAGGAGCTGGAAGG	59.5	317-504
	AGAAGCACCTCTGGTGCCAC		
Genes Assessed in MSCs (Mixed MSC, TNF-MSC1, LPS-MSC1 and ATMSC)			
TRAIL	CGATTTCAGGAGGAAATAAAAGAA	57.6	144
	TCCATATTCTGCATCTTTAGACCA		
FSP	GATGAGCAACTTGGACAGCA	58	127
	CTTCCTGGGCTGCTTATCTG		
FAP	TCAACTGTGATGGCAAGAGCA	60.3	219
	TAGGAAGTGGGTCATCTGGGT		
CXCL12	ATGAACGCCAAGGTCGTGGTC	60	267
	CTTGTTTAAAGCTTTCTCCAGGTACT		
EGF	TGCCAACTGGGGGTGCACAG	58	342
	CTGCCCGTGGCCAGCGTGGC		

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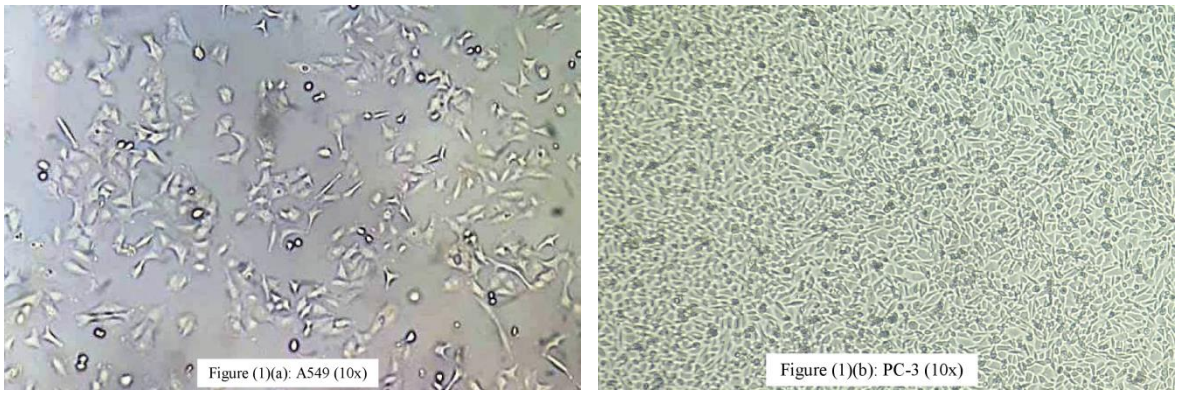
V. RESULTS

A. *Morphology of Cancer Cells*

❖ ***Small Cell Lung Carcinoma (A549) Cells & Prostate Carcinoma (PC-3) Cells***

Both cancer cell lines grew as adherent cells and revealed epithelial like morphology **(Figure 1. (a) & (b)** respectively**).**



(a) Small Cell Lung carcinoma cells (A549) **(b) Prostate carcinoma cells (PC-3)**

Figure (1) (a)(b) Morphology of cancer cells: Both were adherent reflecting epithelial like morphology.

B. *Doubling Time of Cancer Cells*

Doubling time of both the cancer cell lines A549 **(Refer Figure 2. (a) (b) and (c))** and PC-3 **(Refer Figure 3. (a) (b) and (c))** was determined by performing growth curve assay.

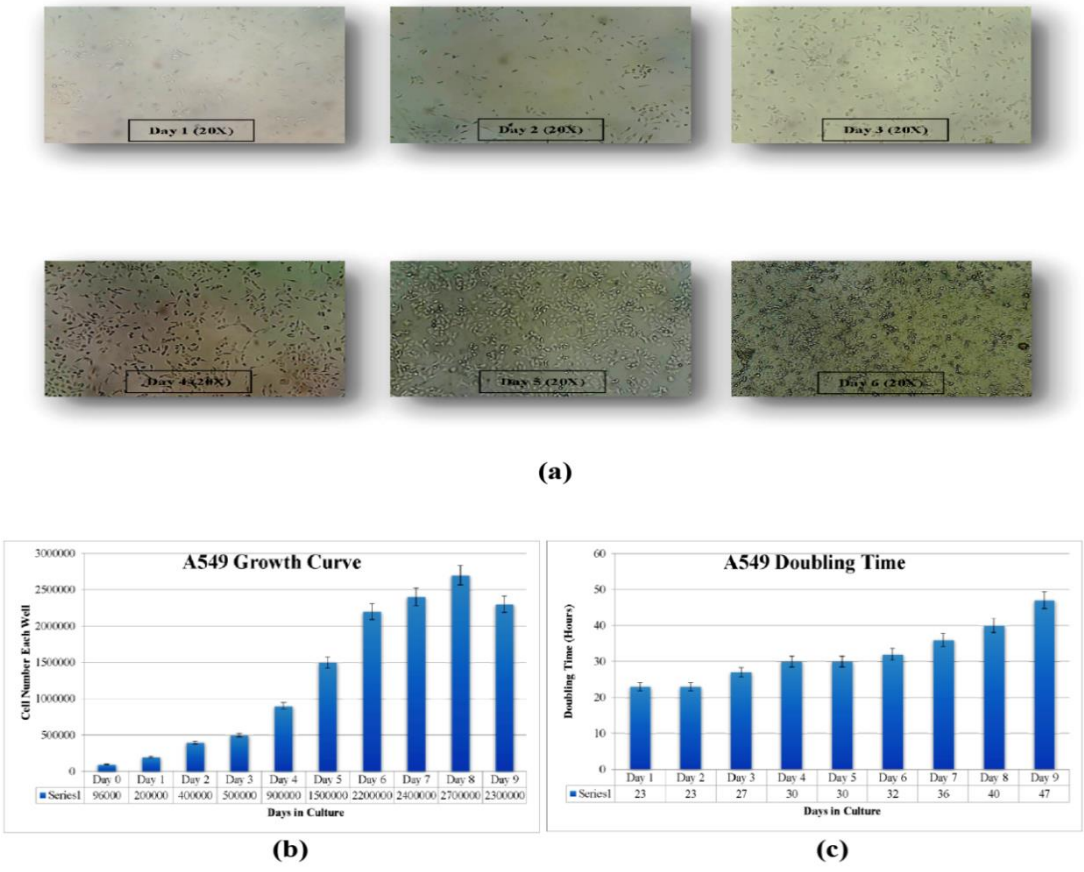


Figure (2) Growth of A549 Cells.

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(a) Pictures of Growth Curve of Lung Carcinoma Cell Line (A549): Each well of the six well plate was harvested every 24 hours and pictures were captured before harvesting and counting.

(b) Growth Curve of Lung Carcinoma Cell Line (A549): Note that the cell number kept elevating exponentially till day 8 and from day 9 started to drop-down.

(c) Doubling Time of Lung Carcinoma Cell Line (A549): The population doubling time (PDT) was calculated on the basis of growth curve assay. The PDT for A549 was 23 hours initially, after which it increased up to 47 hours on day 9. This is probably because of growth slow-down due to lack of space and nutrients. However, ATCC information suggested a PDT of 23 hours. The experiment was carried out in triplicate and results were reported as Mean ± SD.

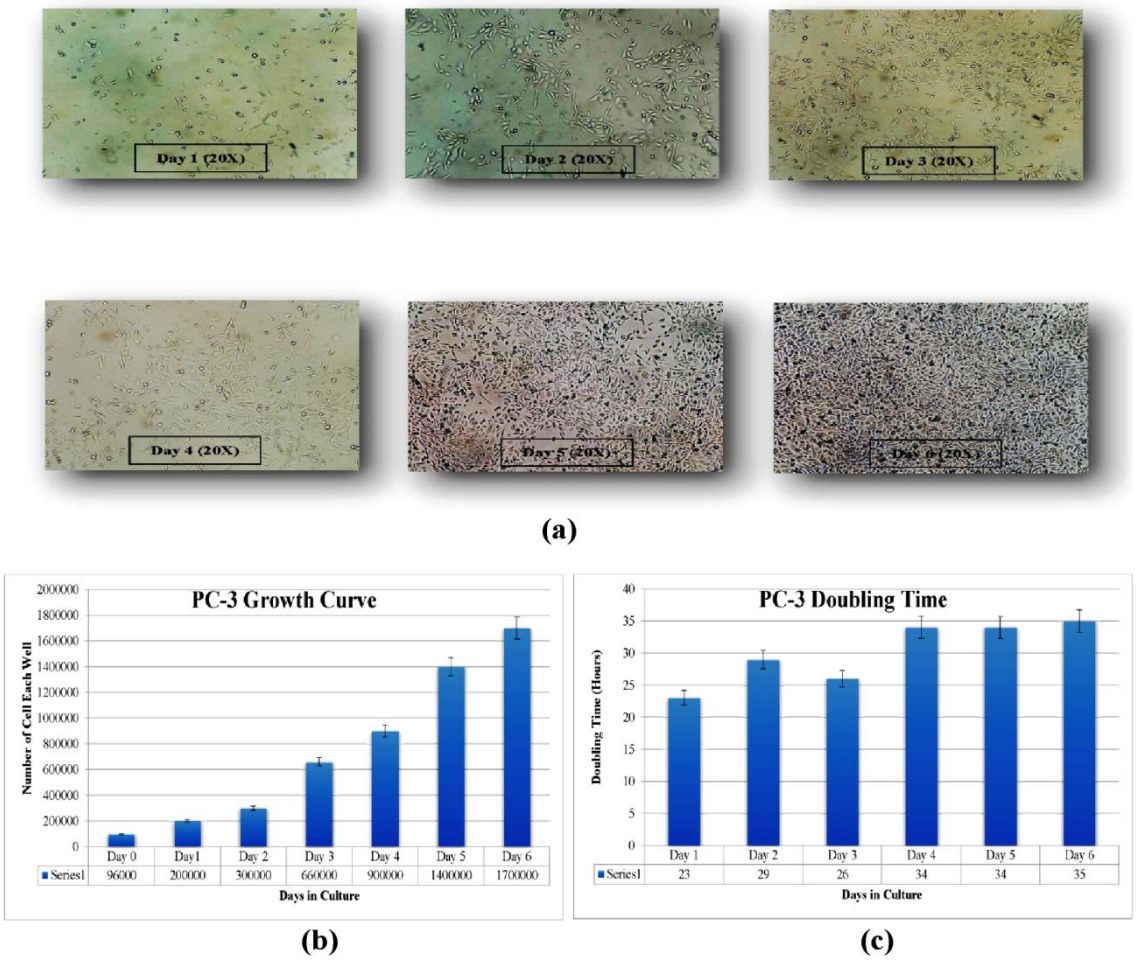

Figure (3) Growth PC-3 Cells.

(a) Pictures of Growth Curve of Prostate Cancer Cell Line (PC-3): Each well of six well plate was harvested every 24 hours and pictures were captured before harvesting and counting.

(b) Growth Curve of Prostate Cancer Cell Line (PC-3): Note that the cell number kept elevating exponentially.

(c) Doubling Time of Prostate Cancer Cell Line (PC-3): The population doubling time (PDT) was calculated on the basis of growth curve assay. The average PDT from day 1 to day 3 was 26 hours and then rose to 35 hours on day 6. The experiment was carried out in triplicate and results were reported as Mean ± SD.

C. Morphology of MSCs – Mixed MSC, LPS-MSC1, TNF-MSC1, ATMSC

Light microscope was used to observe MSCs. Images were captured and recorded at regular frequency. Cells were observed for attachment to plastic surface and morphology as described in literature. MSCs revealed a typical 'fibroblast - like' morphology as shown in **Figures (4)(a) Mixed MSC, Figure (4)(b) LPS-MSC1, Figure (4)(c) TNF-MSC1, Figure (4)(d) ATMSC**

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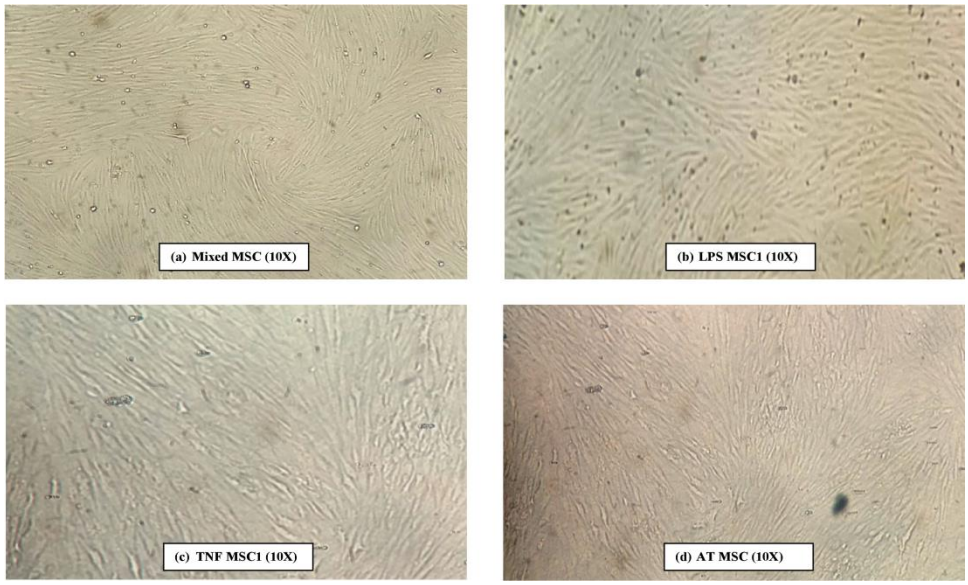
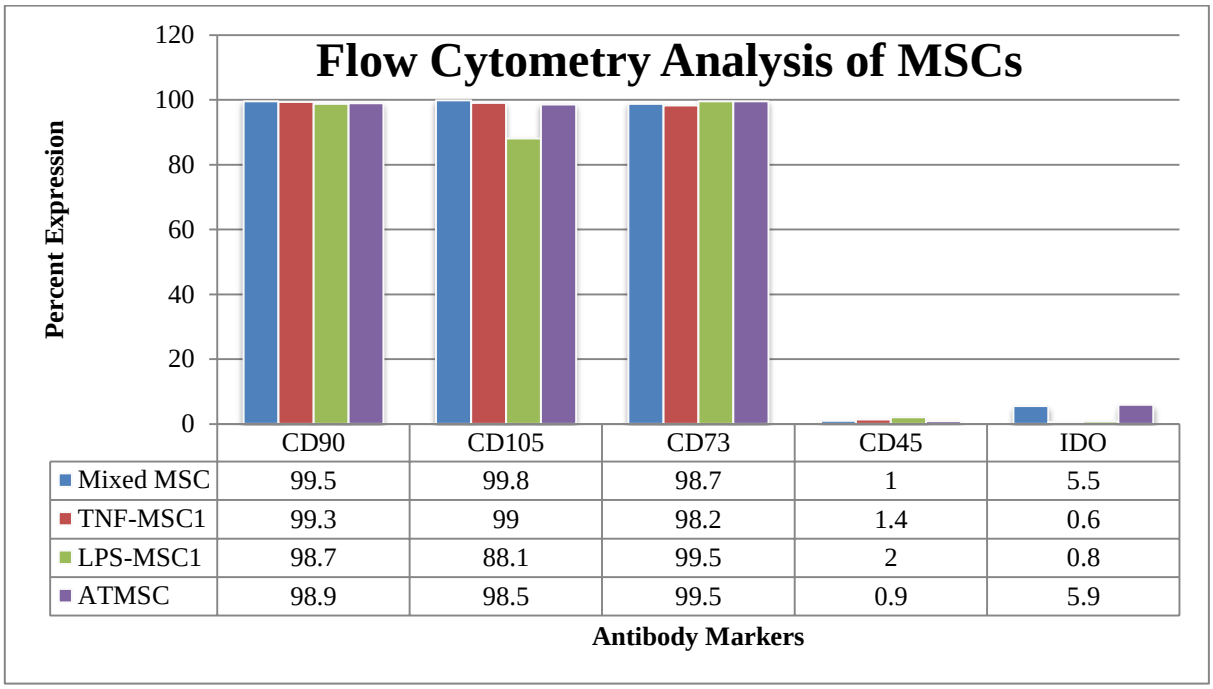


Figure 4) Morphology of MSCs (a) Mixed MSC (b) LPS-MSC1 (c) TNF-MSC1 (d) ATMSC. MSCs formed monolayer and reflected fibroblast like elongated morphology.

D. Establishing Identity by Flow Cytometry

The identity of MSCs was established by flow cytometric analysis. The analysis revealed that the Mixed MSC, very much like MSC1 and ATMSCs expressed typical MSC markers namely CD90, CD73 and CD105 and did not express CD45 **(Figure 5)**. Flow cytometric analysis also showed that IDO initially expressed by Mixed MSCs and ATMSCs at a minimal level was further suppressed by MSC1 as reported earlier [5] [7]. Suppression of IDO expression is indicative of a pro-inflammatory nature of MSC1.



	CD90	CD105	CD73	CD45	IDO
Mixed MSC	99.5	99.8	98.7	1	5.5
TNF-MSC1	99.3	99	98.2	1.4	0.6
LPS-MSC1	98.7	88.1	99.5	2	0.8
ATMSC	98.9	98.5	99.5	0.9	5.9

Figure (5) Immunophenotyping of MSCs (Mixed MSCs, TNF-MSC1, LPS-MSC1 and ATMSC) by Flow Cytometry. Note the cells were strongly positive for Mesenchymal markers CD90, CD73 and CD 105 and negative for hematopoietic markers CD45. IDO expression by Mixed MSCs, ATMSCs, LPS-MSC1 and TNF-MSC1-shows 5.5%, 5.9%, 0.8% and 0.6% respectively.

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E. Co-culture Assay (Cancer cells Vs. MSCs)

Cell Counts

Cell counts of Small cell lung carcinoma cell line A549 after 48 hours of incubation with different types of MSCs **(Figure (6)(a)).** Cell count of A549 in a dish containing positive control Methotrexate (MTX) was comparable with cell counts of A549 with TNF-MSC1 ($p = 0.009$). A549 with ATMSC showed significant difference compared with A549 cell count with TNF-MSC1 ($p = 0.009$). Rest of the cell counts was statistically non-significant. Cell count of PC-3 in a dish containing positive control Methotrexate (MTX) was found reduced to 0.38 X 10^6 cells. However, we did not find any statistically significant difference in rest of the assay sets. (**Figure (6)(b))**

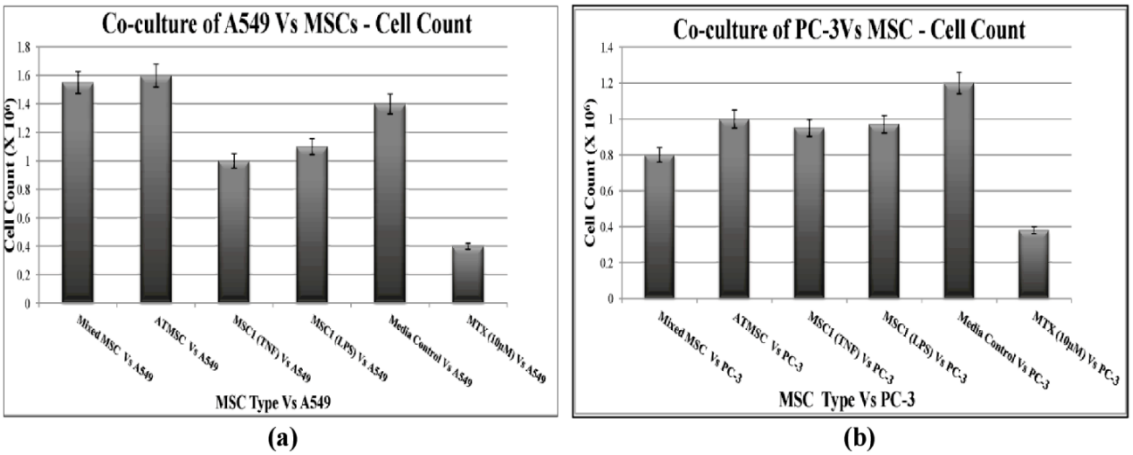

Figure (6) Cell Counts of Cancer Cell Post Assay.

(a) Cell Count of A549 Post-assay. TNF-MSC1 reduced cell count of A549 which was comparable with reduction in cell count by MTX and ATMSC ($p = 0.009$ for both). **(b) Cell Count of PC-3 Post-assay.** The positive control MTX showed reduced cell counts in PC-3 but the remaining sets were almost equal and did not find any statistically significant observation. The experiment was carried out in triplicate and results were reported as Mean ± SD.

F. Apoptosis Assay

The percent apoptosis in A549 by TNF-MSC1 was 8.1% as compared to those induced by Mixed MSC (4.2%, $p < 0.001$), ATMSC (4.1%, $p < 0.001$) and LPS-MSC1 (4.5%, $p < 0.001$) (**Figure (7)(a)).** The percent apoptosis of PC-3 cells induced by TNF-MSC1 was 13.7%, that induced by Mixed MSC was 15.4%, that induced by ATMSC was 11.7%, and that induced by LPS-MSC1 was 10%. These were statistically not-significant (**Figure (7)(b)).**

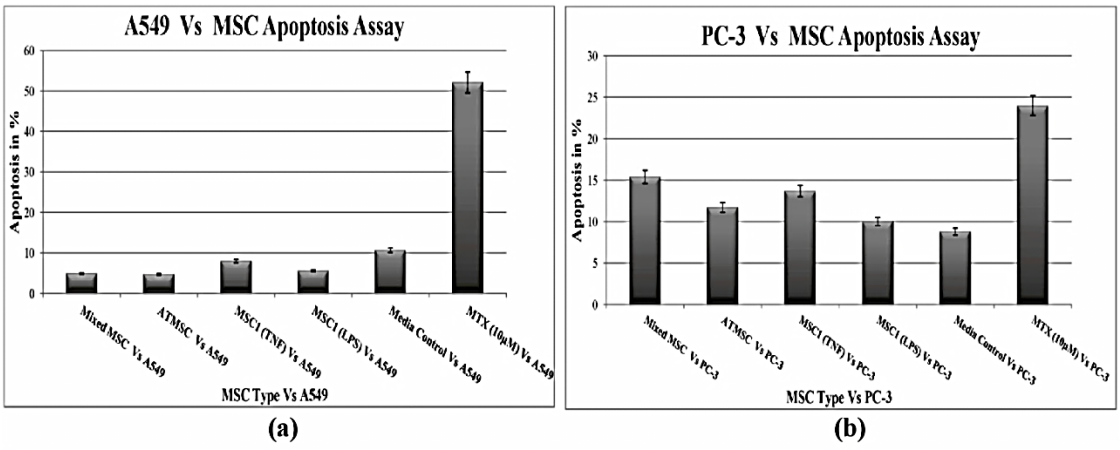

Figure (7) Apoptosis Activity in Cancer Cells Post-Assay.

(a) Apoptosis Activity of Different Types of MSCs on A549. The percent apoptotic activity was higher (8.1%) in A549 treated with TNF-MSC1 in contrast to Mixed MSC, ATMSC and LPS-MSC1 (4.2%, 4.1% and 4.5% respectively; $p < 0.001$ for all).

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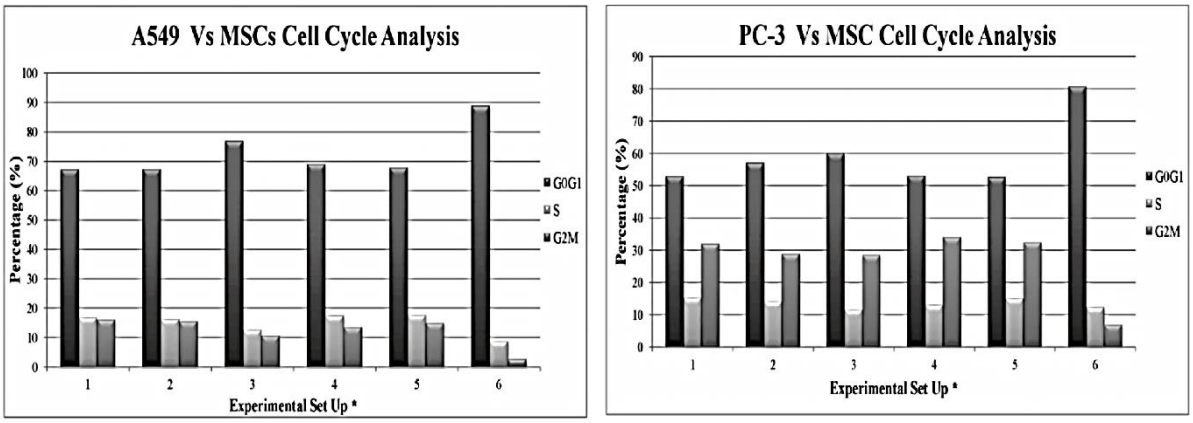
(b) Apoptosis Activity of Different Types of MSCs on PC-3. The percent apoptotic activity was higher (15.4%) in PC-3 treated with Mixed MSC in contrast to TNF-MSC1 (13.7%), ATMSC (11.7%) and LPS-MSC1 (10%) but these differences were statistically non-significant. The experiment was carried out in triplicate and results were reported as Mean ± SD.

G. Cell Cycle Analysis

G0G1 phase of cell cycle was arrested to a certain extent (76.8%) when A549 cells were cultured with TNF-MSC1, as compared to A549 cultured with Mixed MSC (67.2%, $P < 0.001$), with ATMSC (67.3%, $p < 0.001$) and with LPS-MSC1 (69%, $p = 0.001$) (**Figure (8)(a)).** There was no statistical difference between MSCs and PC-3 cells (**Figure (8)(b)**).



(a) **(b)**

Figure (8) Comparison of Cell Cycle Analysis in Cancer Cells Post-Assay.

(a) Cell Cycle A549: Comparison of G0G1 Phase of A549 Cultured with Different Types of MSC. (b) Cell Cycle PC-3: Comparison of G0G1 Phase of PC-3 Cultured with MSCs. There was no statistical difference among different sets except set number 6 (Positive Control). * Experimental set up – 1: Mixed MSC Vs A549; 2: ATMSC Vs A549; 3: TNF-MSC1 Vs A549; 4: LPS-MSC1 Vs A549; 5: No MSC Vs A549 Control; 6: MTX (10 uM) Vs A549 Positive Control. The experiments were carried out in triplicate and results were reported as Mean ± SD.

H. Gene Expression by MSCs & Cancer Cells by RT-PCR

The Tumor Activating Factors studied included FSP (Fibroblast specific protein), FAP (Fibroblast activating protein) and EGF (Epidermal growth factors). Down-regulation of FSP and FAP was observed in Mixed-MSCs after induction with TNF-α and LPS (TNF MSC1 and LPS MSC1). However, FAP was suppressed extensively in LPS-MSC1 (Mixed-MSC induced with LPS) compared to TNF-MSC1 as shown in **Figure (9)(a).**

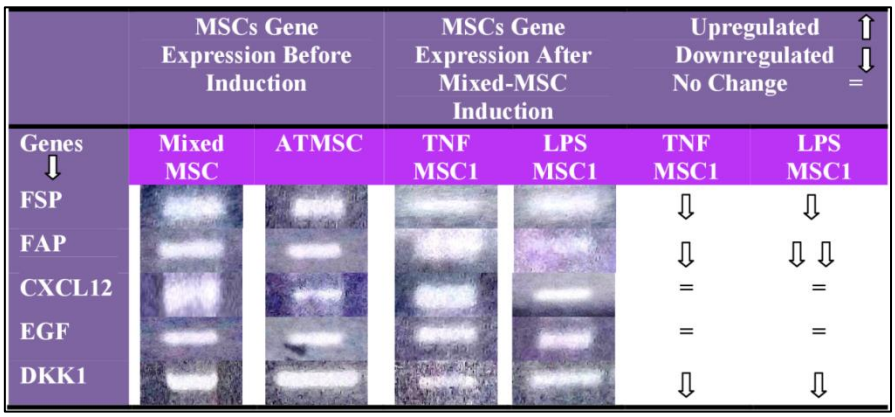


Figure (9)(a) Gene Expression Assessed in MSCs using RT-PCR: The expression patterns of TAFs (Tumor Activating Factors), inhibitor of Wnt signaling (DKK1) and CXCL12 (Soluble protein for cell multiplication signaling) in MSCs before and after induction with TNF-α and LPS was assessed using RT-PCR.

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DKK1, an inhibitor of Wnt pathway was studied in MSCs pre-induction (Mixed MSCs and ATMSC) and post-induction of Mixed MSCs only (TNF-MSC1 and LPS-MSC1) (**Figure (9)(a)**). The expression of DKK1 was down-regulated after an induction with TNF-α and LPS (TNF-MSC1 and LPS-MSC1). The degree of down-regulation was the same in TNF-MSC1 and LPS-MSC1.

The gene expression pattern was the same as shown in **Figure (9)(a)** in MSCs pre-induction (Mixed MSCs and ATMSC) and post-induction of Mixed MSCs only (TNF-MSC1 and LPS-MSC1) for CXCL12, a soluble protein secreted by MSCs and plays crucial role in CXCL12/CXCR4 pathway.

The base level TRAIL (Tumor necrosis factor-Related Apoptosis Inducing Ligand) expression in Mixed MSCs and ATMSCs is shown in **Figure (9)(b)**. TRAIL was expressed by Mixed MSCs and ATMSCs at very negligible levels as evidenced from lighter bands. TRAIL expression was elevated in TNF-MSC1 compared with Mixed MSC and a significantly brighter band was observed in LPS-MSC1 indicating at least three fold elevations in expression level in Mixed MSC induced with LPS [26] [27] [30]. This observation was very significant but on the basis of reduction in cancer cell numbers in co-culture by TNF-MSC1, we checked expression patterns of various genes in cancer cells which were co-cultured with TNF-MSC1 only.

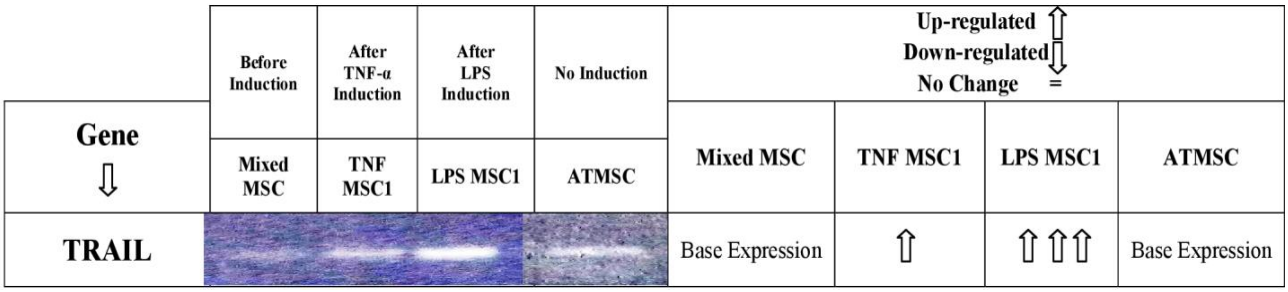

Figure (9)(b) TRAIL Expression Assessed in MSCs by RT-PCR: The expression patterns of TRAIL (Tumor necrosis factor-Related Apoptosis Inducing Ligand), pro-apoptotic gene, in Mixed MSC, ATMSC, TNF-MSC1 and LPS-MSC1 was assessed using RT-PCR.

We looked for expression patterns of tumor suppressor genes like *BAX* (BCL2-Associated X Protein), *BCL2* (B cell lymphoma 2) and *BIRC5* (Survivin) in cancer cells co-cultured with TNF-MSC1 (**Figure (9)(c)**). BCL2 was up-regulated in small cell lung carcinoma cells (A549) and there was no difference of expression levels in prostate carcinoma cells (PC-3). There was no change in expression patterns of BAX gene in both the cancer cell lines (A549 & PC-3). We did not observe any expression of BIRC5 in both the cancer cell lines (A549 & PC-3) before or after co-culture.

The expression patterns of Cyclin D1 and c-Myc, targets of Wnt signaling pathway, w are as shown in **Figure (9)(c)**. Cyclin D1 was down-regulated in A549 cells and up-regulated in PC-3 cells after co-culture with TNF-MSC1. This probably indicates the least anti-cancer response from PC-3 post co-culture, while c-Myc found to be down-regulated in both A549 cells & PC-3 cells after co-culture with TNF-MSC1. The Gene expression patterns of Wnt signaling pathway proteins were as shown in **Figure (9)(c)**. Two genes, β-Catenine and APC were studied. β-Catenine was down-regulated in both A549 cells & PC-3 cells after co-culture with TNF-MSC1. The expression levels of APC gene remained the same in both the cancer cell lines (A549 & PC-3) before or after co-culture with TNF-MSC1 suggesting an anti-tumor effect, if at all, and is probably mediated by β-Catenine and is APC-independent.

DKK1, an inhibitor of Wnt signaling pathway, was down-regulated in A549 cells after co-culture with TNF-MSC1 as shown in **Figure (9)(c)**. However, we did not evaluate its expression in PC-3 cells.

Finally, we studied the expression patterns of CXCR4 and CXCR7, the constituent proteins of CXCL12/CXCR4 axis, in both the cancer cell lines (A549 & PC-3) before or after co-culture with TNF-MSC1 as shown in **Figure (9)(c)**. CXCR4 was down-regulated drastically in A549 cells and there was no change in expression patterns in PC-3 cells. CXCR7, an intracellular protein of CXCL12/CXCR4 axis, did not show any change in expression patterns in both the cancer cell lines (A549 & PC-3) before or after co-culture with TNF-MSC1.

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	Cancer Cells Before Co-culture		Cancer Cells After TNF-MSC1 Co-culture		Up-regulated ⇧ Down-regulated ⇩ No Change =	
Genes ⇩	A549	PC-3	A549	PC-3	A549	PC-3
β - Catenine					⇩	⇩
CXCR4					⇩	=
CXCR7					=	=
C-Myc					⇩	⇩
APC					=	=
Cyclin D1					⇩	⇧
BCL2					⇧	=
DKK1		NA		NA	⇩	NA
BAX					=	=
BIRC5	Not Expressed by Both Cancer Cell Lines					

Figure (9)(c) Gene Expression Assessed in Cancer Cells (A549 & PC-3) using RT-PCR: The gene expression patterns of tumor suppressor genes, targets of Wnt signaling pathway (Cyclin D1 and c-Myc), Wnt signaling pathway proteins (β-Catenine & APC) and an inhibitor of Wnt signaling pathway (DKK1) were studied in both cancer cell lines (A549 & PC-3) before and after co-culture with LPS-MSC1.

VI. CONCLUSION

The objective of the present study was to evaluate the possible intrinsic anti-cancer properties of MSCs derived from perinatal tissues (Mixed MSCs) and to identify the probable mechanisms of action. We modulated 'Mixed MSCs' by priming with TNF-α and LPS to produce TNF-MSC1 & LPS-MSC1 respectively on the lines of papers published earlier [26] [27] [30] and evaluated their anti-cancer potentials on cancer cells in co-culture experiments. Adipose tissue derived MSCs (ATMSCs) were used for the purpose of comparison and to check if it has anti-cancer properties.

TRAIL belongs to TNF family and plays a crucial role of induction of apoptosis in cancer cells [31]. LPS, as reported earlier with bone marrow derived MSCs, results in priming of TLR4 (Toll-like Receptor 4) giving rise to immunosuppressive and an anti-cancer phenotype [26] [27].

TNF-MSC1 exerted significant anti-cancer effect on small cell lung carcinoma cells (A549), while there was no statistically significant anti-cancer effect observed on prostate carcinoma cells (PC-3).

The observations from apoptosis assay were in-line with the trend at least as far as 'cell counts' as a parameter was concerned. The apoptosis induced in A549 cells by TNF-MSC1 was statistically significant as compared to its counterparts. However, there was no statistically significant apoptosis reported in PC-3 cells by TNF-MSC1.

We performed cell cycle analysis of cancer cells after co-culture with MSCs to check if the apoptosis in cancer cells is cell cycle-dependent. Surprisingly, in A549 cells cultured with TNF-MSC1, G0G1 phase of cell cycle was arrested to a relatively smaller extent .as compared with A549 cells cultured with other MSC types. However, PC-3 cell cycle was not arrested at any phase by any type of MSC.

LPS-MSC1 did not exert any anti-cancer effect on either of the cancer cells , perhaps due to the dominance of anti-apoptotic signals from CXCL12/CXCR4 axis, a subject to be researched later.

We shifted our focus on gene regulation pattern in MSCs [basic and induced] with cancer cells before and after co-culture using semi-quantitative RT-PCR. In MSCs, TAF (Tumor activating factors) which contributes to the tumor angiogenesis and invasion, namely FSP (Fibroblast specific protein) and FAP (Fibroblast activating protein) post induction with TNF-α and LPS were down-regulated. However, gene expression pattern by EGF (Epidermal growth factors) did not change.

The TRAIL expression was significantly higher in LPS-MSC1 and in TNF-MSC1 but not as much as LPS-MSC1. This observation was initially surprising since higher expression of TRAIL by LPS-MSC1 did not correlate with the extent of anti-cancer effect exerted by LPS-MSC1 in either of the cancer cells. However, when we checked the expression of CXCR4 by cancer cells, we got satisfactory clarification. CXCR4 was down-regulated in A549 cells after co-culture with

TNF-MSC1, but in PC-3 cells, the expression pattern remained same before and after co-culture. The expression of CXCR7 remained the same in both cancer cell lines before and after co-culture with TNF-MSC1. The down-regulation of CXCR4 in A549 probably explains its TRAIL-dependent response to anti-cancer cues from TNF-MSC1. CXCR4 expression remained unchanged in PC-3 and hence the anti-cancer apoptotic signals may have got overridden leading to no reduction in PC-3 cell numbers in co-culture with TNF-MSC1. CXCL12/CXCR4 signaling pathway was reported to be involved in the homing and multiplication of MSCs and hence could contribute significantly to the cancer metastasis by overriding apoptotic signals [32] [33]. CXCL12/CXCR4 signaling pathway is also actively involved in developing drug resistance, signals for the survival and proliferation of cancer cells, enhances tumor metastasis by regulating angiogenesis and overrides apoptotic signals released by TRAIL (Tumor necrosis factor-Related Apoptosis Inducing Ligand) [33].

Tumor suppressor genes were studied in cancer cells after co-culture with TNF-MSC1 by RT-PCR. The expression remained same for BAX in both cancer cell lines after co-culture with TNF-MSC1. BCL2 found to be up-regulated in A549 post co-culture with TNF-MSC1 and was not changed in PC-3. BIRC5 was not expressed by both the cancer cell line either before or after the co-culture with TNF-MSC1.

Dis-regulation of Wnt signaling pathway reported to be involved in many cancers and associated metastasis and hence we investigated expression patterns of two genes involved in this pathway. β-Catenine found to be down-regulated in A549 and PC-3 but the expression pattern remained same for APC before and after co-culture with TNF-MSC1. Down-regulation of β-Catenine in A549 justifies reduction in cell number post co-culture with TNF-MSC1.

c-Myc and Cyclin D1, the downstream effectors of Wnt signaling, were assessed in cancer cells after co-culture with TNF-MSC1. c-Myc was down-regulated in both cancer cell lines and Cyclin D1 was down-regulated in A549 while was up-regulated in PC-3 post co-culture with TNF-MSC1.

Finally, we studied the expression pattern of DKK1 (Dickkopf-related protein 1), a Wnt signaling inhibitor, in MSCs and cancer cells. DKK1 was down-regulated in TNF-MSC1 and LPS-MSC1 post induction and down-regulated in A549 post co-culture with TNF-MSC1.
However, we could not evaluate the secreted factors in a co-culture system.

VII. DISCUSSION

We are witnessing an era of development of stem cell therapeutics spanning across various domains such as neuromuscular, hematopoietic, genetic and metabolic disorders and autoimmune disorders, thus paving its way to meet several unmet medical needs. The potential of stem cells especially Mesenchymal Stem Cells (MSCs) is promising, owing to its varied clinical application-friendly properties [2]. The multilineage differentiation potential of MSCs equips it with the flexibility to apply this cell type in multiple degenerative disorders including diabetes, neuronal and musculoskeletal origin [34] [35] [36]. These properties are supplemented with immune priviledgeness circumventing the hurdles of immune histocompatibility barriers in clinical settings [2] [37]. MSCs can be isolated from varied sources like bone marrow, adipose tissue and umbilical cord tissue and cord blood. However, MSCs derived from perinatal tissues (umbilical cord blood, placenta and amniotic sac) seems to be more efficient owing to their ontogenetic naivety [7] and is dependable source for unlimited number of MSCs for allogeneic or autologous use for various degenerative disorders [38].

MSCs, slowly but steadily, are being considered as a therapeutic tool in various malignancies [39] [40] [41] [42]. MSCs possess tumor tropism property and hence they are being increasingly considered for use as a vehicle for delivery of anti-cancer agents to the site of tumor [2]. However, MSCs possess intrinsic anti-cancer properties to attenuate growth of several types of cancer cells [39] [40]. Yuan *et al* suggested that proliferation and migration of lung carcinoma and hepatocarcinoma cells was arrested in different phases of cell cycle and exerted apoptotic effect when co-cultured with MSCs derived from cord tissue [39]. Consistent with our observations, the expression of BCL2 (Tumor suppressor gene), β-catenin and c-Myc was down-regulated in this study. Ma *et al* reported anti-cancer effect of umbilical cord tissue derived MSCs on cancer stem cells derived from primary breast cancer cells. They observed anti-cancer effect *in vitro* and *in vivo* and concluded that MSCs derived from cord tissue exerted anti-cancer effect on breast cancer stem cells *in vitro* and *in vivo* probably due to underlying mechanism of induction of apoptosis, cell cycle arrest and suppression of pro-tumor genes and pathways [40].

The intrinsic anti-cancer properties of MSCs may be affected by several factors including source of MSC derivation, cancer cell type under investigation, stage of the cancer, differences in *in vivo* & *in vitro* conditions, age of the patient and some interfering anti-apoptotic signaling pathways like CXCL12/CXCR4 axis.

These outcomes are suggestive of specificity of MSCs towards particular cancer cells with respect to anti-cancer behavior. In the present study, TNF-MSC1 showed tumor cell regression only for small cell lung carcinoma cell lines (A549) and there was no effect on prostate cancer cell line (PC-3) despite the experiments were carried out in the same environmental condition using same cell types derived from single donor and at the same time. We could not find any comparative studies to justify our observations.

We also observed that the source and priming of MSCs has major impact on their anti-cancer behavior. Mixed MSC, LPS-MSC1 and ATMSC did not exhibit any anti-cancer trait on either of the cancer cell lines, however, TNF-MSC1 revealed the same on A549 cells. The other factors which may affect anti-cancer properties of MSCs are the 'in *vivo'* and '*in vitro'* conditions. These observations are in line with observations by Lee *et al* where they pre-incubated MSCs derived from bone marrow with TNF-α and not only seen up-regulation of pro-apoptotic TRAIL but also weekly infusions of these activated MSCs into mice resulted in to regression of lung tumors formed by breast cancer cells (MDA-MB-231) [30]. They also reported the involvement of apoptotic MDA cells to further up-regulate TRAIL in TLR3-depedent manner. However, we speculate that there may not be involvement of CXCL12/CXCR4 axis to exert anti-apoptotic signals which otherwise would have nullified TRAIL apoptotic signals. DKK1, Wnt signaling inhibitor, shown to be up-regulated in this study causing suppression of MDA cell cycling [30]. However, in our study we observed down-regulation of DKK1 in MSCs after induction with TNF-α and LPS. The down-regulation was also observed in A549 cells but still anti-cancer effect was observed in A549 cultured with TNF-MSC1. The underlying mechanism of action remains a topic of further research.

There may be supplementary signaling pathways and cascading signals which cannot be replicated in glass conditions. For example, TNF-MSC1 may act on cancer cells by elevating overall immune response to tumor cells through myriad of other proteins, cells and cascading pathways of the immune system which cannot be replicated in *in vitro* conditions. TNF-α is tumor cytotoxic and acts on tumor by elevating inflammatory response to tumor microenvironment [43]. The difference in the expression of TNF receptors on cancer cell surface may result into variation in the anti-cancer activity in TNF-dependent apoptosis. However, we could not evaluate the behavior of these MSCs in *in vivo* conditions.

TRAIL is the major activator of apoptosis in cancer cells and is secreted by perinatal MSCs to a lesser extent. TRAIL works by imposing apoptosis mediated by complex network of pathways thereby inhibiting DNA synthesis as evident from cell cycle analysis. Methotrexate, a potent chemotherapeutic agent and folic acid analogue, exerts apoptosis by inhibiting nucleotide synthesis in target cells [44]. However, being chemotherapeutic agent, Methotrexate is non-specific and damages normal cell flora around cancer tissue. MSCs may address this issue since MSCs possess tropism for site of tumor and can be used for targeted anti-cancer activity [5]. TRAIL expression up-regulates upon priming with TNF-α and LPS, however, CXCL12/CXCR4 pathway overrides apoptotic signals exerted by TRAIL thereby leaving TRAIL ineffective. As evident from the results, perinatal MSCs (Induced and non-induced) reportedly do not transform into tumor associated fibroblasts (TAFs) which confers safety compared with MSCs derived from bone marrow [45]. MSCs derived from bone marrow reportedly transform into tumor associated fibroblasts (TAFs) when grown near breast cancer microenvironment and thereby plays critical role in tumor metastasis [45]. This observation was consistent *in vivo* and *ex-vivo* as well. However, MSCs derived from perinatal tissue like Wharton's Jelly didn't transform into TAFs. This virtue is an added advantage as far as safety of MSCs is concerned when it comes to possible clinical application.

This is further supported by up-regulation of tumor suppressor genes like BCL2 which result in suppression of oncogene. Wnt signaling proteins and their downstream target genes were down-regulated when cultured with TNF-MSC1. The up-regulation of tumor suppressor genes is an essential activity to avoid metastasis and to impose apoptotic signals on cancer cells. The tumor suppressor gene *BAX* remained unchanged in both the cancer cell lines; the reason for the same can be investigated further. We feel the need for a dedicated study to study expression of tumor suppressor genes in MSCs and cancer cells after co-culture to find the best suitable MSCs.

Though, in the present study we tried to find anti-cancer activities of native and primed MSCs, there are many unanswered questions for which future research is to be undertaken. We tested only two cancer cell lines and were unable to study other cancer cells. A pilot study testing various types of cancer cells against different types of MSCs probably will throw some light in this direction. For example, TRAIL mediated apoptosis is reported in glioma cells through MSCs derived from bone marrow [46], however, the role of bone marrow derived MSCs in glioma metastasis was not studied in this research. They observed that the anti-cancer effect was mediated by inhibition of angiogenesis. The role of different MSCs in cancer angiogenesis remains to be investigated further. It is of particular importance since angiogenesis plays crucial role in cancer metastasis. The role of perinatal & primed MSCs in Epithelial-Mesenchymal (EMT) transition could be a starting point of investigation even before performing studies involving angiogenesis. EMT serves as an initial indicator of transformation of MSCs into cancer cells, if at all involved in metastasis. Further, why A549 only shown anti-tumor effect and PC-3 did not remain to be investigated. Priming of MSCs with LPS resulted into greater expression of TRAIL compared with that of TNF-α, however, anti-tumor activity exerted by TNF-MSC1 was greater than LPS-MSC1; this question demands further studies at a molecular level. The detailed role of CXCL12/CXCR4 axis was not investigated in this study. The hypothesis that in PC-3 cells, CXCL12/CXCR4 axis might have exerted anti-apoptotic signals on TRAIL in co-culture with LPS-MSC1 was not evaluated by using CXCL12/CXCR4 axis blocking analogues. However, we recommend use of CXCL12/CXCR4 axis blocking analogues to obtain optimal activity of TRAIL thereby causing apoptosis in cancer cells. Use of blocking analogues *in vivo*, however, is non-specific and could pose risk of blocking this essential pathway in normal cells surrounding tumor microenvironment. We also propose detail oriented pre-clinical animal studies to observe and understand if *in vitro* observations remain consistent *in vivo*. We feel that answering these gaps will bring us little more closure to the concrete conclusion in near future by performing follow up studies.

In summary, the outcome of the present study suggest that TNF-MSC1 may exert TRAIL-dependent anti-cancer effect on small cell lung carcinoma cells (A549) in *in vitro* mediated by apoptosis and/or cell cycle arrest and by inhibiting Wnt signaling pathway. It also proved the hypothesis that the MSCs from different sources possess anti-cancer properties perhaps for a particular cancer cell type only. In short, anti-cancer behavior of MSC is very specific to source of MSCs and cancer type under investigation. Additionally, we appreciate the complexity of different signaling pathways that support anti-cancer properties exerted by MSCs. MSCs were used as vehicle for targeted anti-cancer drug delivery, however, we demonstrated that the MSCs, once primed, up-regulates TRAIL secretion and causes apoptosis in cancer cells. However, to deal with anti-apoptotic signals by CXCL12/CXCR4 axis, we hypothesize that the use of 'CXCL12 or CXCR4 antagonists' along with TNF-MSC1 or LPS-MSC1 may be useful for the effective anti-cancer effect [33].

This study shows the probable usefulness of modified MSCs in cancer therapeutics. The mechanisms and hypothesis assumed in this study need to be researched further utilizing multiple approaches. This will and should encompass further cancer cell types and employ quantitative analysis to make the research translatable.

CONFLICT OF INTEREST

The Authors do not have any conflict of interest.

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