Characterization of tumor-derived mesenchymal stem cells potentially differentiating into cancer-associated fibroblasts in lung cancer

Characterization of Tumor-derived mesenchymal stem cells potentially differentiating into Cancer-Associated Fibroblasts: a case report study in lung cancer.

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Abstract

Purpose: The goal of this study was to understand if mesenchymal stem cells isolated from lung tumor tissue (T-MSCs) may differentiate into Cancer Associated Fibroblasts (CAFs), that promote neoplastic progression, angiogenesis and metastasis in the epithelial solid tumors, mimicking the tumor microenvironmental influence.

Methods: MSCs were been obtained from healthy (Control, C-MSCs) and tumor (T-MSCs) tissue of one patient who underwent a lobectomy for a lung adenocarcinoma pT1bN0. Isolated cells were characterized for the presence of molecular markers (identified by routine diagnostic characterization in differentiated tumoral cells), stemness properties, and CAF-related markers expression. Subsequently, cells were co-cultured with a lung adenocarcinoma cell line (A549 cells) to evaluate the effects on proliferation, oncogene expression and IL6 secretion.

Results: C- and T-MSCs did not present EGFR mutations unlike tumor tissue and showed a stem-like immunophenotype, characterized by the ability to differentiate towards osteo-, chondro- and adipogenic lineages. The expression of markers referred to CAFs (α-SMA, HI-1α, MMP11, VEGF, CXCL12, TGF-β1, TGF-βRII, IL6, TNFα) was significantly higher in T-MSCs than in C-MSCs. The co-cultures with A549 cells led to the over-expression of selected oncogenes and to the increase of IL6 secretion in T-MSCs but not in C-MSCs.

Conclusions: MSCs isolated from tumor tissue displayed distinct properties compared to MSCs isolated from healthy tissue, suggesting T-MSCs differentiation towards a CAF-related phenotype under the influence of the tumoral microenvironment.

Keywords: lung cancer; mesenchymal stem cells; cancer associated fibroblasts; IL6

Introduction

Lung cancer represents the major cause of cancer-related death worldwide [1]. Historically, lung cancer has been subdivided in two different histological groups: small cell and non-small cell lung cancer (SCLC and NSCLC). NSCLC is the most frequent type and can be further subdivided into squamous cell carcinoma (SCC), adenocarcinoma, and large cell carcinoma [2].

While increased understanding of the genetic landscape of lung cancer has led to the identification of druggable targets and improved molecular stratification of the patients, the contribution of non-tumor cells (i.e. tumor-stroma interaction) to the onset and progression of the disease still remains unclear.

Mesenchymal stem cells (MSCs) are multipotent cells that can influence tumor progression through the secretion of soluble factors or direct interaction with epithelial cells, promoting survival, proliferation and cell migration. As precursors of most stromal cells, their involvement in carcinogenesis has been suggested for different tumor types, including lung cancer, but the precise effects of MSCs on lung tumor cells is still controversial [3]. Among various hypotheses, carcinoma-associated fibroblasts (CAFs) have been suggested to originate from MSCs [4]. These cells represent the major component of tumor stroma, acting as myofibroblasts, and can be identified using alpha-smooth muscle actin (alpha-SMA) as a biomarker [5]. CAFs are thought to promote tumor growth and are generally associated with poor prognosis [6]; therefore, identification of CAFs derived from lung MSCs may pave the way to potential therapeutic opportunities in lung cancer [7].

In this work, we describe a case report of a patient diagnosed with early-stage lung adenocarcinoma who underwent surgery intervention. Mesenchymal stem cells (MSCs) were isolated and characterized at a histological and molecular level, to better shed light on lung MSC properties and possibly devise novel treatment strategies.

Material and Methods

Human tissue collection and molecular analysis

Tumor and healthy samples of lung were collected from a 52-year old woman who underwent a left lower lobectomy and systematic nodal dissection at the “Ospedali Riuniti” of Ancona (Italy). The preoperative staging revealed clinical T2N0 disease, indicating anatomic lung resection as the ideal therapy for this patient. The lesion was positive (SUV 7.9). No preoperative chemo- and radio-therapy was delivered. The final pathologic staging reported adenocarcinoma pT1bN0. Due to the early stage of disease no further medical therapy was proposed.

Genomic DNA (gDNA) was extracted from formalin-fixed paraffin-embedded tissue from the left pulmonary lesion and underwent routine diagnostic molecular characterization for EGFR, KRAS, NRAS, PIK3CA, BRAF, ERBB2, ALK, DDR2, MAP2K1, RET mutations using Myriapod Lung Status (Diatech Pharmacogenetics) by MassARRAY Sequenom analysis.

Ethical approval

The patient provided her written informed consent to participate in the study, which was approved by the institutional ethics committees (Prot. 2016 0360 OR) and was conducted in accordance with the Declaration of Helsinki.

Cell culture

One healthy and one tumoral tissue fragment was placed into 6-well plates containing MSCGM medium (Lonza, Basel, Switzerland). Cell morphology was evaluated by phase-contrast microscopy (Leica DM IL; Leica Microsystems GmbH, Wetzlar, Germany) and viability by an automated cell counter (Invitrogen, Milano, Italy). MSCs isolated from healthy lung were used as Control (C-MSCs), while MSCs isolated from tumor lung were defined T-MSCs.

Mutational profiling of candidate genes in tumor tissue and MSC cells.

PCR and sequencing primers were designed using Primer 3 program and synthesized by Sigma (Online Resource 1). PCR primers were designed to amplify the selected exons (EGFR exon 18-19-20-21) and the flanking intronic sequences, including splicing donor and acceptor regions. A touchdown PCR program was used for PCR amplification and all samples were subjected to Sanger Sequencing by automated ABI PRISM 3730 (Applied Biosystems). Sequence traces were analyzed using the Mutation Surveyor software package (SoftGenetics, State College, PA).

Droplet digital PCR (ddPCR)

Isolated gDNA was amplified with ddPCR Supermix for EGFR p.T790M, p. L858R and p.E746_A750del assays. ddPCR was performed according to the manufacturer's protocol (Bio-Rad) and as described by Siravegna and colleagues [8].

Doubling time

To assess doubling-time, $8x10^4$ cells/well were plated using an algorithm available online (http://www.doubling-time.com): DT = t x lg2 / (lgNt – lgN0) where N0 is the number of plated cells, Nt is the number of harvested cells, and t is culture time in hours [9].

Characterization of C- and T-MSCs

Cells were characterized by testing plastic adherence [10], immunophenotype and multipotency [11]. Briefly, for immunophenotyping, 2.5×10^5 cells were stained for 45 min with fluorescin isothiocyanate (FITC)-conjugated antibodies (Becton-Dickinson) against the indicated protein.

For differentiation assay, cells were induced towards osteocytes, chondrocytes and adipocytes using STEMPRO® Osteogenesis, Chondrogenesis and Adipogenesis Kits (GIBCO, Invitrogen,) respectively [12].

The expression of stemness genes (*OCT4, SOX2, NANOG, KLF4*) was analyzed by Real Time PCR (RT-PCR) as previously described [12].

mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method [13], where ΔCt=Ct (gene of interest)—Ct (control gene) and Δ (ΔCt)=ΔCt (T-MSCs)—ΔCt (C-MSCs). The list of primers is reported in Online Resource 2.

Analysis of the expression of α-SMA by Western blot

α-SMA expression was tested by Western blot as previously described [11]; membranes were incubated with primary anti- α-SMA antibody (Santa Cruz Biotechnology, Heidelberg, Germany, 1:400) followed by incubation with a secondary antibody conjugated to horseradish peroxidase. Immunoreactive proteins were visualized using a chemiluminescent substrate (Santa Cruz Biotechnology). GAPDH (Santa Cruz Biotechnology) was the endogenous normalizer; Normal Human Lung Fibroblasts (NHLFs, Lonza) and cells from myometrium were the negative and the positive controls respectively. Densitometric analyses of the immunoreactive bands (quantified as α-SMA /GAPDH bands in corresponding samples and expressed as arbitrary units, A.U.) was performed by using UVITEC Imaging Systems (Cleaver Scientific Ltd, Warwickshire, United Kingdom).

Analysis of CAF-associated genes

The expression of selected genes referred to neoplastic progression (HIF1α), invasiveness (MMP11), angiogenesis (VEGF, CXCL12), EMT (TGF-β1, TGF-βRII) and metastasis (IL6, TNFα) was analyzed by RT-PCR in C- and T-MSCs to test the hypothesis of differentiation towards CAFs.

As control, NHLF cells were used. mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method as above mentioned, where ΔCt=Ct (gene of interest)—Ct (control gene) and Δ (ΔCt)=ΔCt (T-MSCs or C-MSCs)—ΔCt (NHLF). The list of primers is reported in Online Resource 2.

Indirect MSCs/A549 co-culture

To test the paracrine effect exerted by MSCs on tumor cells, an adenocarcinoma human alveolar basal epithelial cell line (A549, ATCC® CCL-185™) was co-cultured with C- and T-MSCs for 72h. $1x10^5$ of each sample of MSCs cells were individually seeded at the lower surface and the day after, 1 $x10^5$ A549 cells were added at the upper surface of a polycarbonate transmembrane filter in a Transwell filter system in a 6-well plate (pore size 0.4 μm; BD Falcon). After co-cultures, tumour cells were recovered and cell number assessed by an automated cell counter.

Analysis of tumorigenesis-associated genes

The expression of selected genes involved in specific cellular mechanisms, such as oncogenesis (cJUN, cFOS, cMET) and EMT induction (ACVR1) was tested by RT-PCR in A549 cells before and after co-cultures with C- and T-MSCs. The primer sequences are reported in Online Resource *2*.

mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method, where ΔCt=Ct (gene of interest)—Ct (control gene) and Δ (ΔCt) = ΔCt (A549 co-cultured)—ΔCt (A549 mock) as described above.

IL6 secretion measurement

The secretion of IL6 was investigated by ELISA test (IL6- Human ELISA Kit, Qiagen) in A549 cells before and after co-culture with C- and T-MSCs, as previously described [13].

Statistical analysis

Independent samples t-test was used to evaluate statistically significant differences between samples. Statistical significance was analysed for data from at least three independent experiments. P values less than 0.05 were considered significant.

Results

Cell isolation and proliferation analysis

Cells were isolated from normal and tumor tissue samples of a lung adenocarcinoma patient that underwent therapeutic surgery. Microscopic analysis indicated that both C-MSCs and T-MSCs exhibited a fibroblast-like morphology (Fig.1a). Up to the second passage, the cell population was heterogeneous; afterwards, cells appeared homogeneous by both morphological and cytofluorimetric analysis.

Doubling time was calculated for C- and T-MSCs from passage 1 to passage 8 (21 days); even if the DT was always higher in T-MSCs than C-MSCs (mean 42.68 ± 7.20 hours and 56.72 ± 9.90 hours respectively for C- and T-MSCs), the difference was statistically significant only at passage 6 (Fig.1b).

Sequencing analysis and identification of germline small nucleotide variants

Routine diagnostic sequencing analysis was performed on genomic DNA (gDNA) obtained from FFPE tumor tissue. An EGFR exon 19 E746_A750 deletion, one of the most commonly observed mutations in patients with non-small cell lung cancer [14], was identified.

At a molecular level, and to interrogate for the presence of differentiated tumor cells in the isolated population, the same EGFR genetic alteration was investigated, initially by Sanger sequencing and then by a more sensitive technology using digital PCR (ddPCR). In both cases, the EGFR exon 19 E746_A750 deletion was not detected.

EGFR mutational analysis was also extended to the complete sequence of exons 18-19-20-21, which represent mutation hotspots in NSCLC patients [15]. To identify any potential somatic change, tumor DNA from T-MSC and DNA from the matched normal were screened in parallel. No EGFR mutations were detected by Sanger or ddPCR analysis, but Sanger sequencing analysis identified a synonymous single-nucleotide polymorphism in EGFR exon 20 (c.2361G>A, p.Q787Q) that is registered in the COSMIC database (Mutation ID COSM1451600). This variant was identified as a homozygous germline variant present in both normal (C-MSC) and tumor (T-MSC) DNA, and was detected when the genome of this patient was compared to a reference genome (Figure 2).

Cell characterization

According to the criteria by Dominici, MSCs adhered to plastic and were strongly positive (more than 90% of positive cells, both for C- and T-MSCs) for CD73, CD90, and CD105 and negative for HLA-DR, CD14, CD19, CD34 and CD45 (Online Resource *3*).

C- and T-MSCs were capable of differentiating towards osteogenic, chondrogenic, and adipogenic lineages (Fig.3a-d), without appreciable differences.

Both cell types expressed NANOG, OCT4, SOX2 and KLF4; only KLF4 displayed a statistically significant increase in C-MSCs compared to T-MSCs (Fig.3e).

α-SMA expression

Western blotting and densitometric analysis, in which cells from myometrium and NHLFs were used as positive and negative controls respectively, showed that T-MSCs expressed three times more α-SMA compared to C-MSCs (Fig.4 a,b).

Analysis of genes referred to tumorigenesis and CAFs

The expression of MMP11, TGF-β1, TGF-βRII, IL6, TNFα, HIF-1α, VEGF and CXCL12 was significantly higher in T-MSCs versus C-MSCs and NHLF, suggesting that T-MSCs acquired pro-tumorigenic properties, similar to those reported for CAFs (Fig. 5).

MSCs and A549 co-culture and effects on gene expression

C- and T-MSCs were indirectly co-cultured with A549 cells. The expression of selected genes was analyzed in A549 cells before (mock) and after co-cultures. Co-cultured A549 cells exhibited significantly ($p<0.05$) higher levels of cJUN, cFOS, ACVR1 respect to mock A549 samples. The variations of cFOS and ACVR1 were stronger in co-cultures with T-MSCs than in co-culture with C-MSCs (Fig. 6a). A549 proliferation was not affected by co-culture with C- or T MSCs (data not shown).

Expression profile of inflammatory cytokines

Since at mRNA level the expression of IL6 was significantly higher in T-MSCs than in C-MSCs (Fig. 5), secretion of IL6 was evaluated in A549 cells before and after the co-cultures with C- and T- MSCs.

Compared to mock samples, A549 cultured with C-MSCs produced lower amounts ($p<0.05$) of IL6 than A549 cells co-cultured with T-MSCs (Fig. 6b).

Discussion

Lung cancer is one of the major causes of cancer-related deaths worldwide [1]. In the attempt to find new therapeutic approaches, as for other solid tumors, great attention has been paid to the role of stem cells. It has been reported that mesenchymal stem cells (MSCs) [16], under the tumor microenvironment influence, may differentiate into Cancer

Associated Fibroblasts (CAFs). CAFs are implicated in tumor biology, driving mechanisms such as neoplastic progression, angiogenesis and metastasis. In this scenario, CAFs deserve more consideration and may become new targets for therapy. Their origin is still debated and it is likely that they have more than one precursor cell. Among others, MSCs are considered an attractive candidate. Here, MSCs from tumoral (T-MSCs) and healthy control (C-MSCs) tissues were isolated from a lung cancer patient and, interestingly, they displayed distinct properties.

Molecular analysis on tissue and cellular samples suggested that isolated cells may have mesenchymal origin. Routine diagnostic analysis performed on tumor tissue identified the EGFR E746_ A750del variant which characterizes almost 50% of NSCLC patients and is associated with sensitivity to the small-molecule kinase inhibitor gefitinib [17]. From a genetic prospective, we looked for EGFR-mutant differentiated cells in the isolated cells by taking advantage of two different methods, Sanger sequencing and ddPCR. The latter one can reach very high sensitivity levels, being able to detect as low as one mutant copy among 20,000 copies of normal DNA. None of the two analyses detected the presence of the EGFR E746_ A750del in either C-MSC or T-MSC cells.

Afterwards, since lung cancer may present high level of intratumor heterogeneity and more EGFR mutations may coexist in the tumor [18], we extended our molecular analysis to other exons (18, 20 and 21) of the EGFR tyrosine kinase domain, which include genetic alterations, such as EGFR L858R and T790M, associated to response and resistance to common tyrosine kinase inhibitors [17]. We were not able to identify any EGFR genetic variation, except for a germline single-nucleotide polymorphism in EGFR exon 20, p.Q787Q. This variant has not been shown to have any predictive or prognostic value in lung cancer [19]. The results of this molecular analysis led us conclude that cell populations isolated from normal or tumor biopsies did not contain EGFR-mutant differentiated cells.

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We next proceeded with analysis of mesenchymal stemness markers. Both cell types satisfied the criteria identified by Dominici [10]; therefore, to further elucidate differential characteristics, the expression of gene related to stemness was analyzed by RT-PCR. While the expression of NANOG, SOX2 and OCT4 was comparable between C- and T-MSCs, expression of KLF4 was significantly higher in T-MSCs than in C-MSCs. This result is in line with Lau et al. [20], describing CAFs as exhibiting higher KLF4 expression.

The analysis cell doubling time indicated that T-MSCs had a higher proliferation rate than C-MSCs. MSCs usually exhibit a greater proliferation rate than fibroblasts and this suggests that T-MSCs might be differentiating towards CAFs. To test this hypothesis, the expression of selected CAF-related markers was analyzed. The most widely used marker for CAFs is α-SMA [21]. T-MSCs showed higher expression of α-SMA compared to C-MSCs, and this difference was confirmed by densitometric analysis. An alternative hypothesis about the origin of CAFs is that resident tissue fibroblasts are the precursor cells [22]. Expression of other gene candidates was tested using NHLF as control. It is reported that

CXCL12 and HIF-1α are upregulated in CAFs [23]via the involvement of TGF-β1. We observed TGF-β1 overexpression in our T-MSCs, confirming this trend. CXCL12 plays a pivotal role in the interaction between tumor cells and differentiating CAFs, and has been shown to promote tumor growth and angiogenesis [24]. Angiogenesis superintends development and progression of tumor cells and it is reported that CAFs produce elevated amount of angiogenic factors, such as VEGF. VEGF is the master promoter of angiogenesis (also under the influence of IL6) and its expression reached higher levels in T-MSCs compared to C-MSCs and NHLF. IL6 is a multifunctional cytokine that, in a tumoral microenvironment, acquires important role in cancer progression, migration and angiogenesis. IL6 is predominantly expressed in CAFs compared to resident fibroblasts and, here we detected higher IL6 expression in T-MSCs than in C-MSCs and NHLF. Another role ascribed to CAFs is to promote invasion and metastasis, two processes involving epithelial to mesenchymal transition (EMT), by the secretion of molecules able to degrade the extracellular matrix. Among these, and under the control of CXCL12 [24], the members of the metalloproteinase family (MMPs) cover a crucial role. The expression of MMP11 was significantly higher in T-MSCs than in C-MScs and NHLF. TGF-β1, in addition to being the promoter of CXCL12 and HIF-1α, constitutes an important EMT player and is overexpressed in T-MSCs. These results support the hypothesis that T-MSCs, but not C-MSCs, are differentiating in CAFs. Next, we performed co-culture experiments with the A549 cells to mimic the crosstalk between potential CAFs and tumor cells. At a molecular level, the expression of cFOS and cJUN was significantly higher in co-cultured cells, in particular in co-culture with T-MSCs respect to C-MSCs. These molecules form the activating protein-1 (AP-1) transcription factor that regulates cell growth in different tumor models [25]. Also, expression of ACVR1, a member of TGF-beta superfamily, was increased after co-cultures with T-MSCs, thus underlining the potential CAF-related properties of T-MSCs and their involvement in EMT.

Finally, the level of secreted IL6 was increased after co-cultures with T-MSCs. Previous studies reported that CAFs increase the malignant potential of NSCLC by means of a number of factors, including IL6. In addition, IL6 contributes to lung cancer's resistance to chemotherapy [26] and its level in blood has also been suggested as a prognostic marker for survival in patients with advanced NSCLC treated with chemotherapy [27,28]. Our results agree with the observations of Shintany [29] that found increased expression of IL6 in CAFs compared with lung normal fibroblasts, and that the conditioned media from CAFs (that is comparable to indirect co-cultures system) induced EMT and resistance to cisplatin in NSCLC cells through IL6-mediated signaling.

From a clinical and molecular point of view and considering this specific patient, in case of unfortunate disease relapse, she might be the ideal candidate for chemotherapy plus targeted therapy with EGFR tyrosine kinase inhibitor drugs. This treatment might be effective towards the primary differentiated EGFR-mutant tumors cells but could have potentially low impact on the stroma counterpart containing EGFR wild type IL6-secreting CAFs. The interaction between EGFR

tyrosine kinase inhibitors and wild-type EGFR CAFs should be further investigated at other levels (transcriptomic, proteomic), to understand the clinically relevant mechanisms of resistance to this therapy for about 25% of patients with EGFR mutated adenocarcinoma [30].

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Figure legend

Fig 1 Cell morphology and Doubling time (a) Phase-contrast images of MSCs from control tissue (C-MSCs) and from tumoral tissue (T-MSCs) after 14 days of culture. Scale bar, 100 μm. (b) Doubling time was calculated over 21 days (8^{th} passage). Data are mean±SD;. * $p<0.05$ T-MSCs versus C-MSCs

Fig 2 Identification of a germline SNP in patient's genome Chromatograms of the sequence of part of EGFR exon 20 in reference genome (top panel), patient's tumor gDNA (T-MSC, middle panel) and patient's matched normal gDNA (C-MSC, bottom panel). The arrows indicate the location of the SNP (c.2361G>A, p.Q787Q)

Fig 3 Multilineage differentiation of C- and T-MSCs and expression of genes related to stemness Representative images of differentiation experiments. Osteogenic differentiation: Alizarin red (a, Scale bar: 200 μm); chondrogenic differentiation: acid mucopolysaccharide coloration with Safranin-O (b, Scale bar: 200 μm) and Alcian blue (c, Scale bar: 200 μm); adipocyte differentiation: Oil red staining (d, Scale bar: 100 μm). No differences were noted between C- and T-MSCs. (e) Selected markers of self-renewal and differentiation potential (OCT4, SOX2, NANOG, KLF4) were evaluated by RT-PCR. The expression levels measured in T-MSCs are considered as X-fold with respect to C-MSCs (considered as =1). Data are mean±SD over three independent experiments in triplicates. * $p<0.05$ T-MSCs versus C-MSCs

Fig 4 Western blot and densitometric analyses of α-SMA expression (a) representative Western blot figures showing the bands of α-SMA and of the endogenous control GAPDH; (b) densitometric analyses of the immunoreactive bands (quantified as α-SMA / GAPDH bands in corresponding samples and expressed as arbitrary units, A.U.). Data are mean±SD of three experiments performed in C-MSCs, T-MSCs, NHLF (negative control), cells from myometrium (positive control). * $p<0.05$ T-MSCs versus C-MSCs

Fig 5 Expression of genes refereed to CAFs. Selected markers referred to different tumorigenic mechanisms (invasion: MMP11; EMT: TGFβII, TGFβRII; metastasis: IL6, TNFα; tumorigenesis: HIF-1α; angiogenesis: VEGF, CXCL12) were evaluated by RT-PCR. The expression levels measured in C- and T-MSCs are considered as X-fold with respect to NHLF (considered as =1). Data are mean±SD over three independent experiments in triplicates. * $p<0.05$ C- and T-MSCs versus NHLF; § $p<0.05$ T-MSCs versus C-MSCs

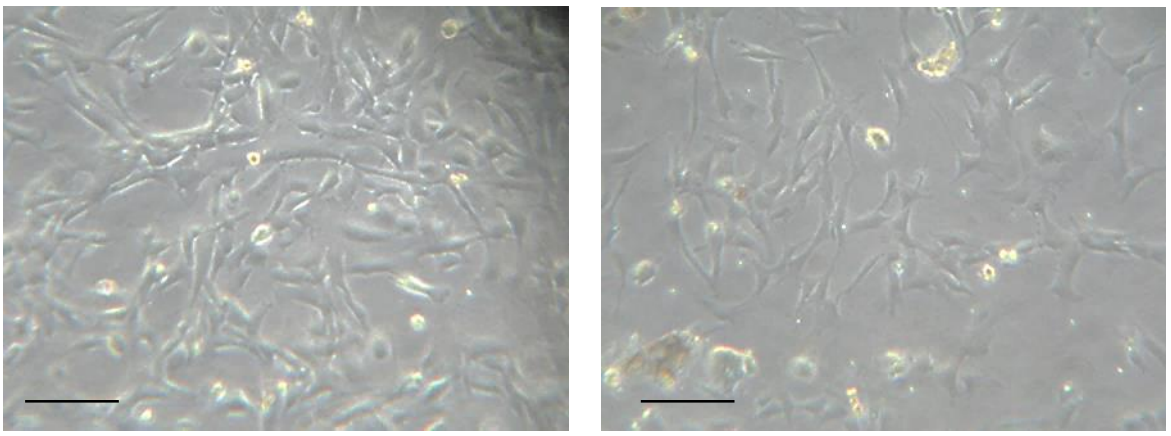
Fig 6 Expression of selected genes by Real Time-PCR (a) and ELISA test (b) (a) The expression of selected genes related to specific cellular mechanisms, such as oncogenesis (cJUN, cFOS, cMET), EMT induction (ACVR1) was detected in A549 cells after co-cultures with C- and T-MSCs. Data are reported as relative expression (X-fold) respect to mock sample (A549 alone, referred as 1). The values of the relative expression of genes of interest are referred as mean ± DS, over three independent experiments in triplicates. *: $p<0.05$ A549 cells versus co-cultured A549. §: $p<0.05$ A549 co-cultured with T-MSCs versus A549 co-cultured with C-MSCs. (b). Secretion of IL6 was detected by ELISA test in C-

MSCs and T-MSCs before and after co-cultures with A549. Tests were performed as triplicates and the levels of secreted cytokine are reported as mean ± SD and expressed as pg/ml. *: $p<0.05$ A549 cells versus co-cultured A549. §: $p<0.05$ A549 co-cultured with T-MSCs versus A549 co-cultured with C-MSCs

a

C-MSCs

T-MSCs



b

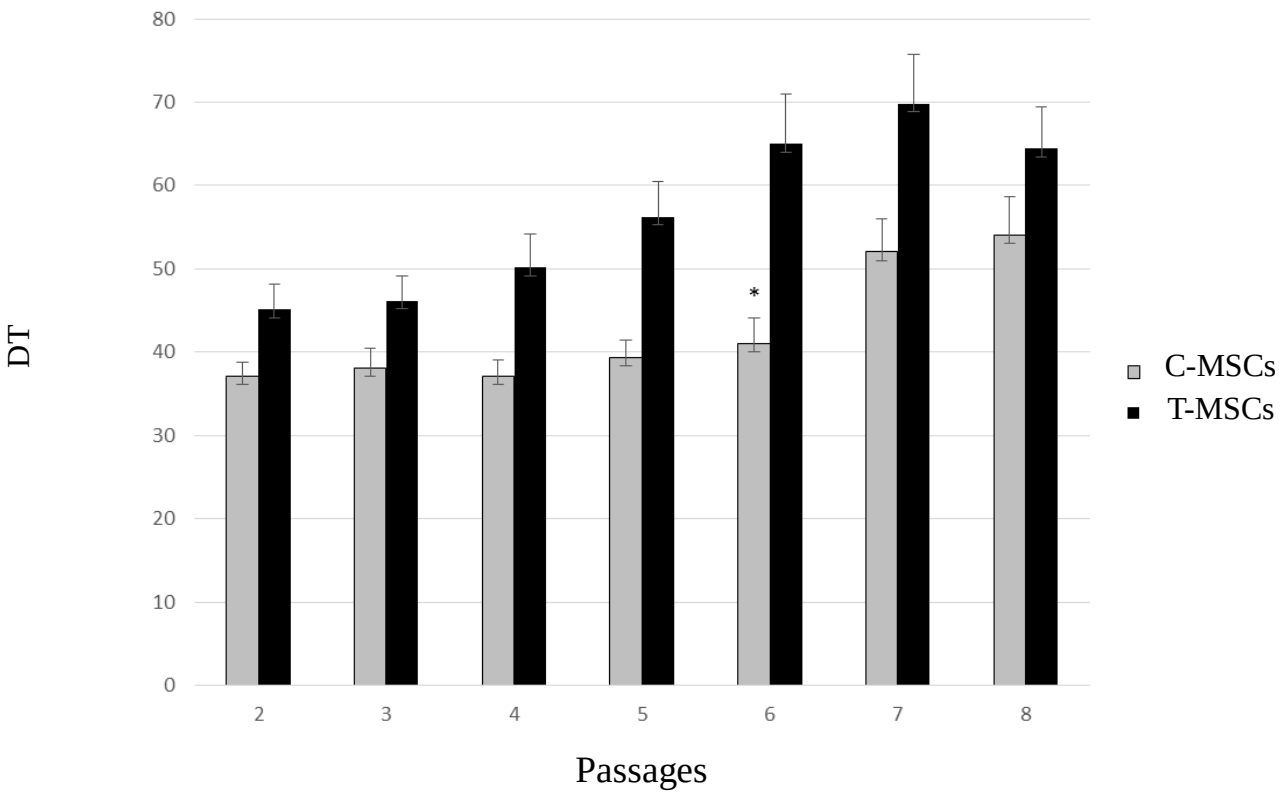


Figure 1

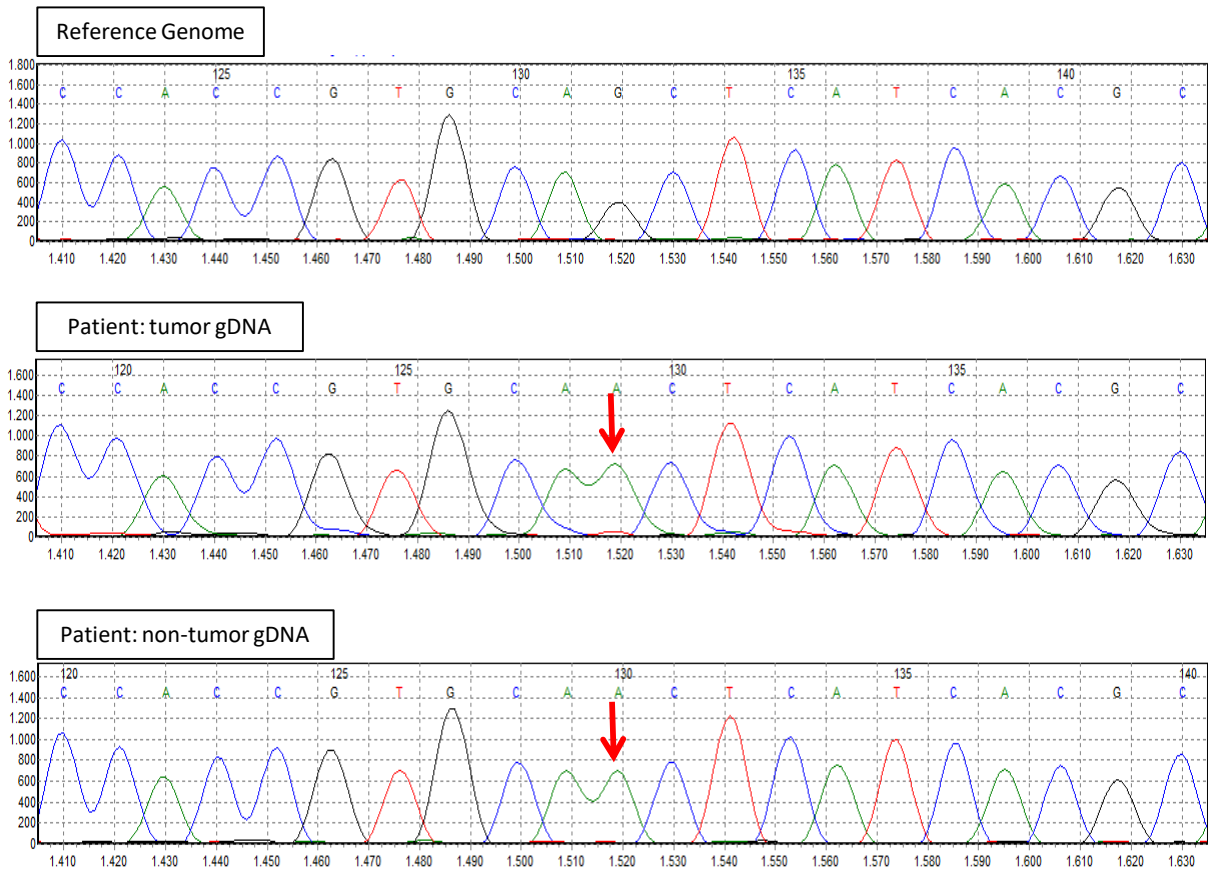
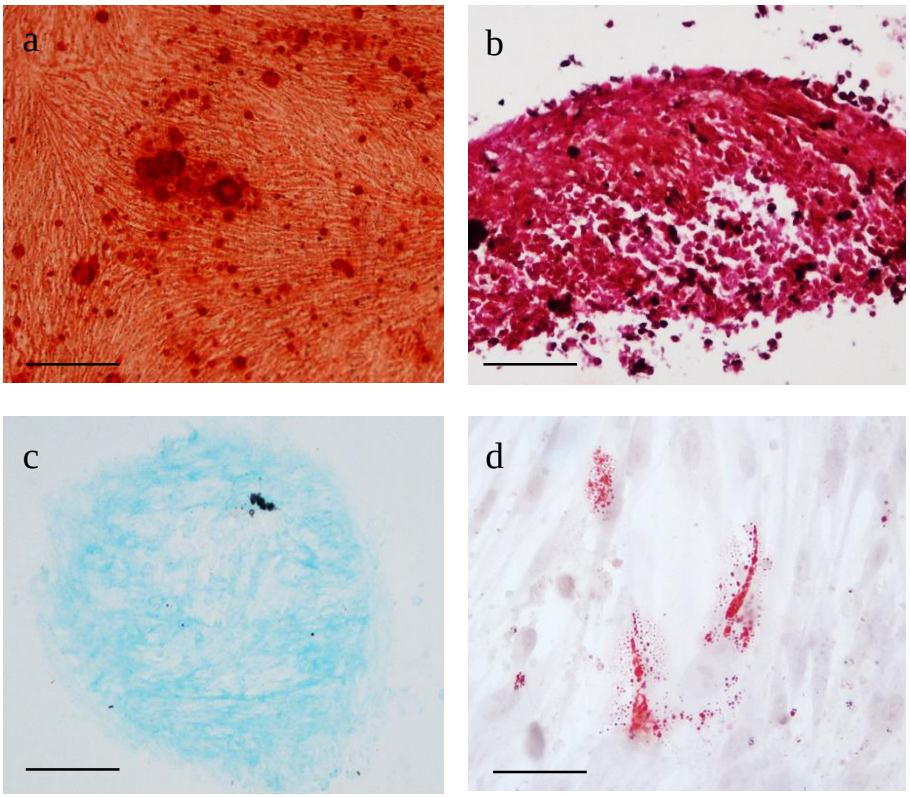

Reference Genome
Patient: tumor gDNA
Patient: non-tumor gDNA

Figure 2


a
b
c
d

e

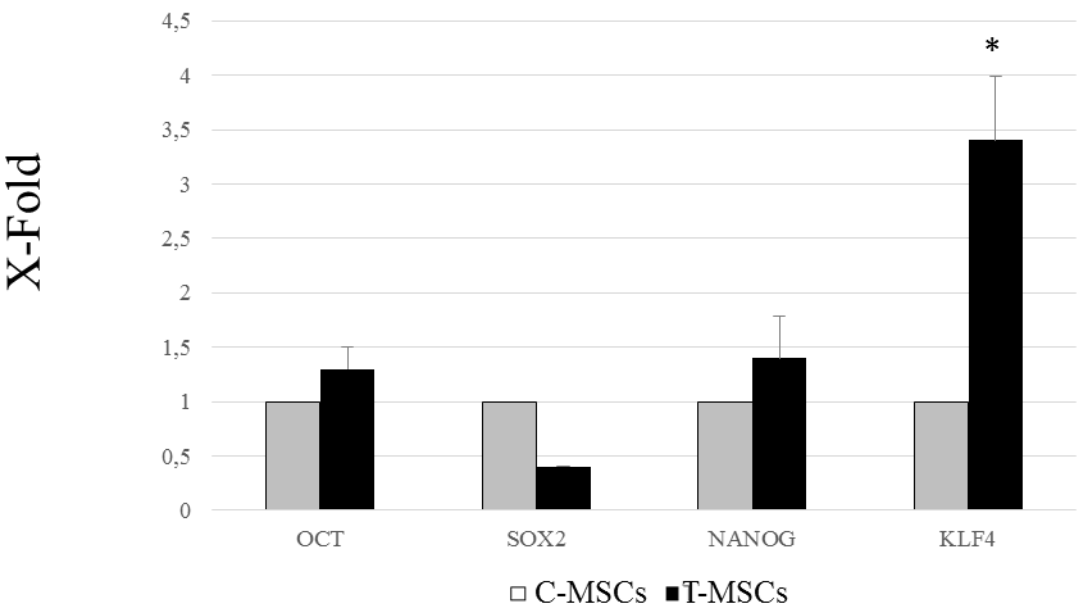
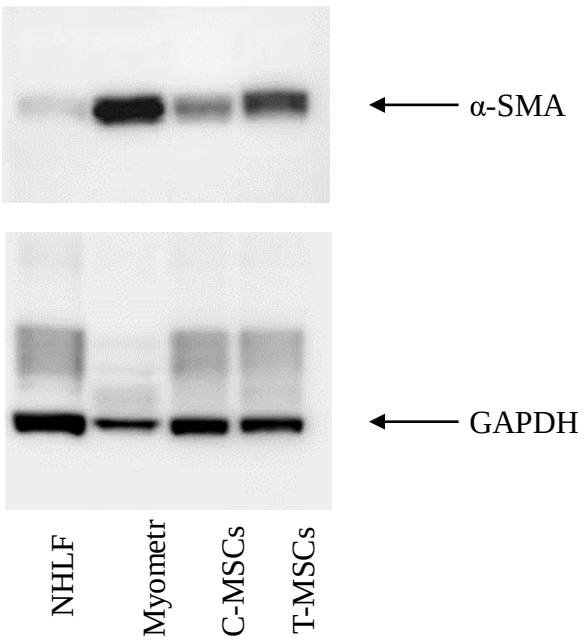

X-Fold
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
*
OCT
SOX2
NANOG
KLF4
C-MSCs
T-MSCs

Figure 3

a


α-SMA
GAPDH
NHLF
Myometr
C-MSCs
T-MSCs

b

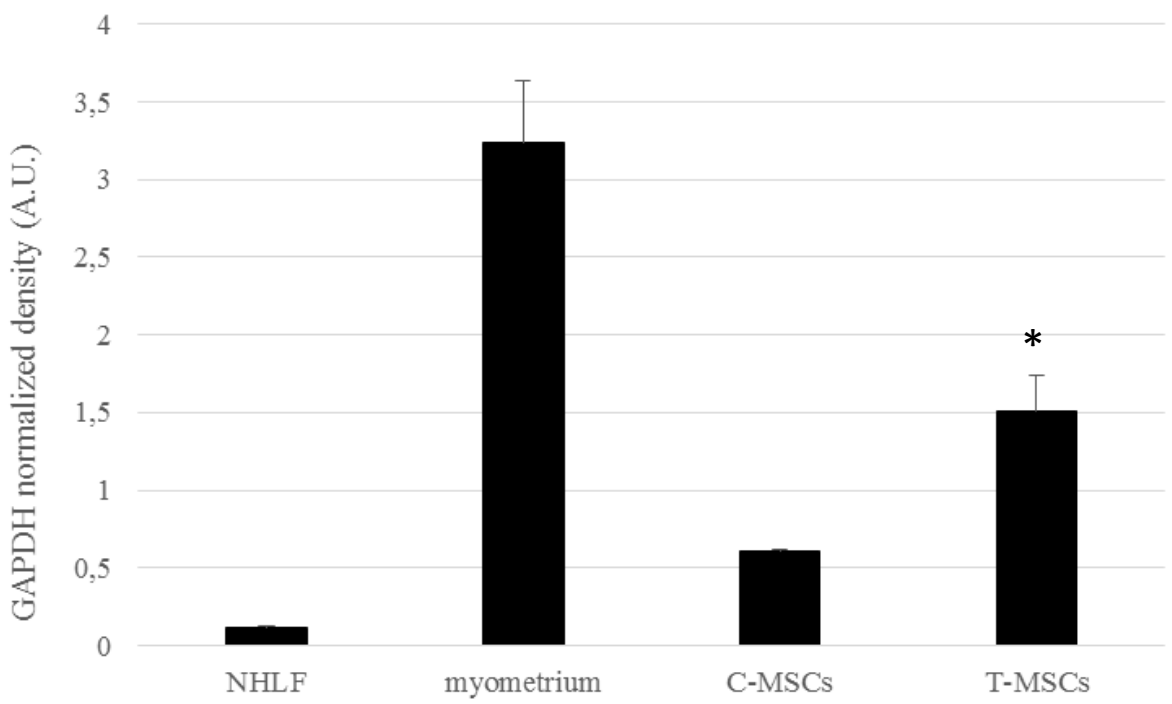

GAPDH normalized density (A.U.)
4
3,5
3
2,5
2
1,5
1
0,5
0
*
NHLF
myometrium
C-MSCs
T-MSCs

Figure 4

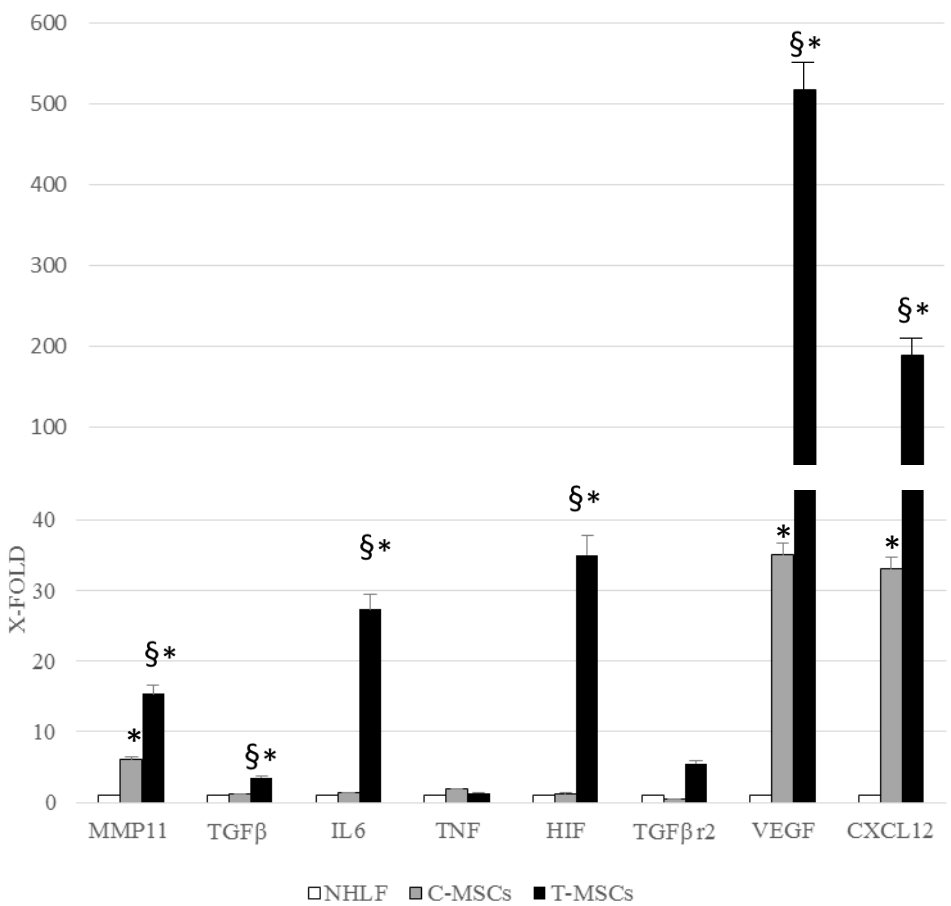
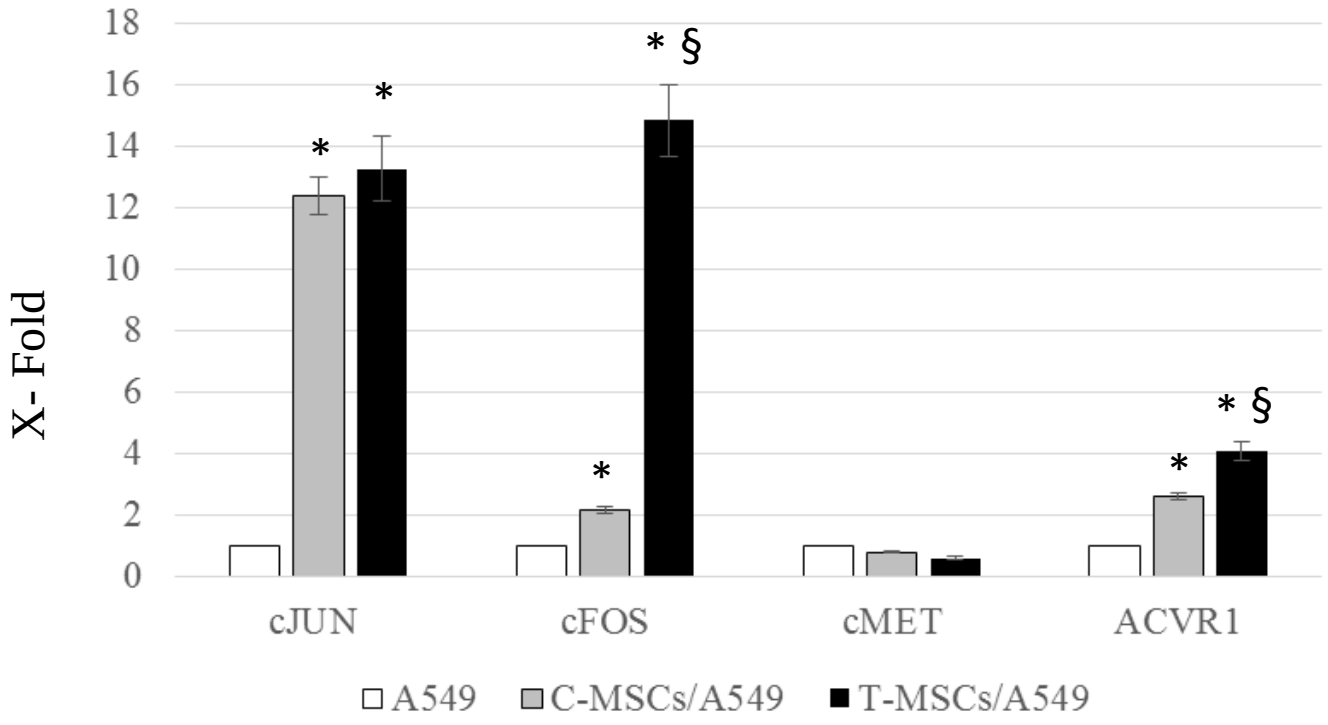

600
500
400
300
200
100
40
30
20
10
0
X-FOLD
§*
*
§*
§*
§*
§*
*
*
MMP11
TGFβ
IL6
TNF
HIF
TGFβr2
VEGF
CXCL12
NHLF
C-MSCs
T-MSCs

Figure 5

a


18
16
14
12
10
8
6
4
2
0
X- Fold
* §
*
*
*
* §
cJUN
cFOS
cMET
ACVR1
A549
C-MSCs/A549
T-MSCs/A549

b

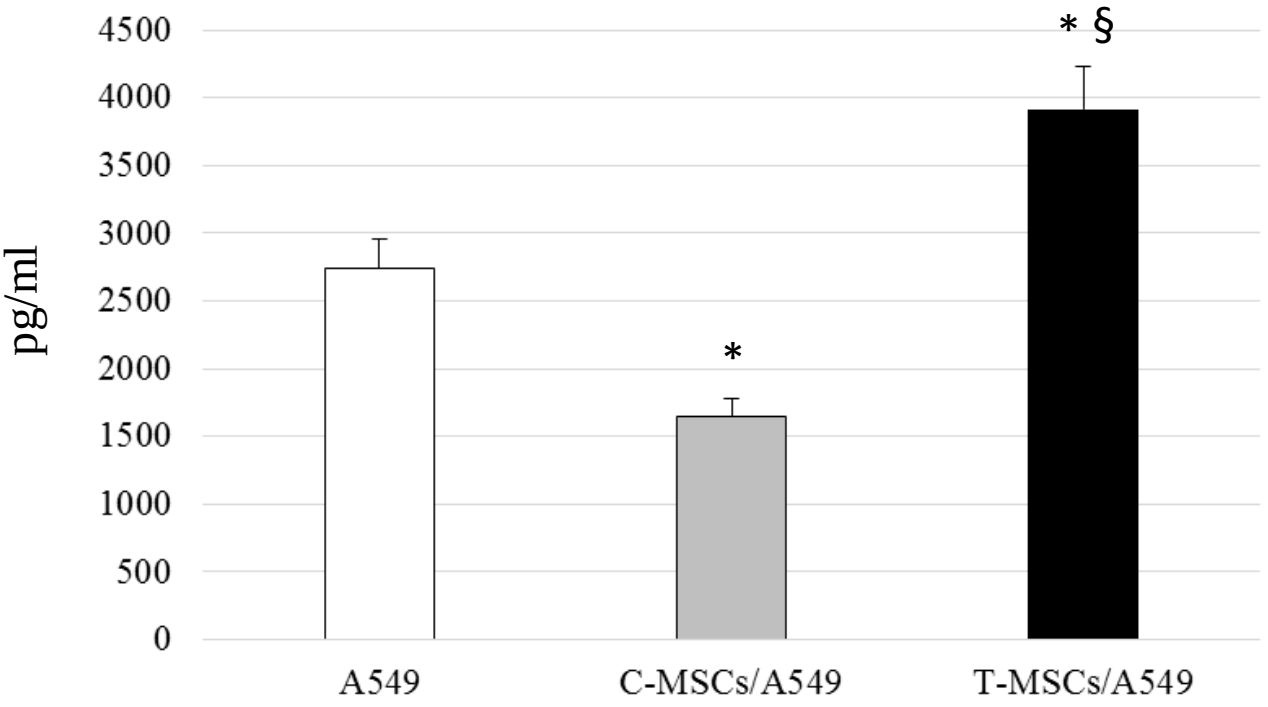

4500
4000
3500
3000
2500
2000
1500
1000
500
0
pg/ml
* §
*
A549
C-MSCs/A549
T-MSCs/A549

Figure 6