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Original Articles

Attenuation of the pro-inflammatory signature of lung cancer-derived mesenchymal stromal cells by statins

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ABSTRACT

Solid tumor growth triggers a dynamic host response, which recapitulates wound healing and defines the tumor microenvironment (TME). In addition to the action of the tumor cells themselves, the TME is maintained by a myriad of immune and stromal cell-derived soluble mediators and extracellular matrix components whose combined action supports tumor progression. However, therapeutic targeting of the TME has proven challenging because of incomplete understanding of the tumor-host crosstalk at the molecular level. Here, we investigated the crosstalk between mesenchymal stromal cells (MSCs) and primary cancer cells (PCCs) from human squamous cell lung carcinoma (SCC). We discovered that PCCs secrete CCL3 and stimulate IL-6, CCL2, ICAM-1 and VCAM-1 expression in MSCs and that the MSC-PCC crosstalk can be disrupted by the lipid-lowering drug simvastatin, which displays pleiotropic effects on cell metabolism and suppresses IL-6 and CCL2 production by MSCs and CCL3 secretion by PCCs. In addition, simvastatin inhibited spheroid formation by PCCs and negatively affected PCC survival. Our observations demonstrate that commonly used statins may be repurposed to target the TME in lung carcinoma.

1. Introduction

Malignant tumor growth invariably triggers a host response, which although highly variable in intensity depending on the tumor type and individual host tissue properties, is composed of the same processes that underlie wound healing, namely, inflammation and tissue remodelling [1–6]. Whereas inflammation may precede tumor growth and is increasingly recognized to be the cause of divergent cancer types [2,4,7], tumor progression both sustains and relies on inflammation and the associated tissue turnover. Exit from dormancy of metastatic cancer cells depends on tissue remodelling triggered by injury, aging or hormonal signals [8–10] and, similarly, transition from carcinoma in situ to invasive tumors may be facilitated by stromal cues elicited by tumor cells [11,12]. Thus, the tumor microenvironment (TME) plays a central role in shaping tumor progression and substantial effort has been invested into developing strategies to divert its tumor-enhancing functions toward neutralization or inhibition of tumor growth. However, success of such approaches in the clinic has been limited because just like a healing wound, the TME is the result of a multitude of complex cellular interactions whose mutual regulatory effects are incompletely understood. Furthermore, the redundancy of the mediators involved, which reflects the importance of inflammation as a vital defense mechanism, renders targeting of any single mediator unlikely to have decisive impact.

Uncovering ways to instruct pleiotropic host tissue cells that modulate the inflammatory response to tip the equilibrium toward its resolution may be a worthy pursuit in an effort to restrain TME support of tumor growth. However, achievement of such a goal will most likely require reagents that target metabolic cellular functions whose modulation may temper the production and/or effect of a range of pro-tumorigenic mediators.

Cells that possess potent pleiotropic properties in the TME include mesenchymal stem/stromal cells (MSCs), a heterogeneous cell population with regenerative potential that can display divergent immunomodulatory activity after priming by pro-inflammatory cytokines or signals triggered by toll-like receptor (TLR) stimulation [13–18]. Mesenchymal stromal cells have been shown to affect the fate of tumor cells in a variety of cancer types and to display Janusian effects as illustrated by their ability to exert both pro- and anti-tumorigenic activity [19–24]. Recent evidence suggests that MSCs in the TME of lung cancers may have a potent effect on the promotion of tumor growth and dissemination [19,25–28].

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Abbreviations

MSCs	Mesenchymal stromal cells (N-MSCs : Normal-tissue-derived MSCs; T-MSCs : Tumor-derived MSCs)
PCCs	Primary cancer cells
SCC	Squamous cell carcinoma
NSCLC	Non-small cell lung carcinoma
TME	Tumor microenvironment
CCL3	Chemokine (C–C motif) Ligand 3 (= MIP-1α: Macrophage inflammatory protein 1-alpha)
CCL2	Chemokine (C–C motif) Ligand 2 (= MCP-1: Monocyte Chemoattractant Protein-1)
CCL4	Chemokine (C–C motif) Ligand 4 (= MIP-1β: Macrophage inflammatory protein 1-beta)
CCL5	Chemokine (C–C motif) Ligand 5 (= RANTES: Regulated on activation, normal T cell expressed and secreted)
PTGS2	Prostaglandin-Endoperoxide Synthase 2
PGE2	Prostaglandin E2
TGF-β	Transforming growth factor-beta
ICAM-1	Intercellular Adhesion Molecule 1
VCAM-1	Vascular cell Adhesion Molecule 1
PDL1	Programmed death-ligand 1 (= CD274)
HMOX-1	Heme oxygenase 1
IL	Interleukin
TLR	Toll-like receptor
TKIs	Tyrosine kinase inhibitors
RCTs	Randomized controlled trials
LPS	Lipopolysaccharides
HLA-E	MHC class I antigen E
HMG-CoA reductase	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
TNF-α	Tumor necrosis factor-alpha
MMP	Matrix metalloproteinases
Bcl-2	B-cell lymphoma 2
BIM	Bcl-2-like protein 11
IFN-γ	Interferon-gamma
NK cells	Natural Killer cells

Lung cancer was among the most commonly diagnosed malignancies in 2018 (11.6% of the total cases) and the leading cause of cancer-related death (18.4% of the total cancer deaths) in both sexes combined [29]. Its most common aetiology is tobacco smoking, accounting for more than 80% of cases. Lung cancer is stratified into a number of subtypes, with approximately 80% of cases belonging to the non-small cell lung cancer (NSCLC) class [30], associated with an overall five-year survival rate of less than 20% (American Cancer Society). The need for more effective strategies for the prevention and treatment of lung cancer is beyond discussion.

Similar to many diverse carcinomas, NSCLC are associated with a prominent TME, which generates numerous soluble mediators that orchestrate the inflammatory and immune response and regulate tumor development. The strong association between inflammation and cancer is reflected, among others, by high IL-6 levels in the TME, where it promotes tumorigenesis by regulating several hallmarks of cancer, including survival, proliferation, angiogenesis, invasion and metastasis, and, perhaps most importantly, metabolism [28,31–35]. Previous reports, including from our group, have shown high production of diverse cytokines by the tumor stroma, with IL-6, being among the most prominent. MSCs are a major source of IL-6, which is a key mediator of innate immunity and favors tumor dissemination [19,26]. In NSCLC, we demonstrated IL-6 secretion by MSCs obtained from primary tumors, referred to as tumor-derived MSCs (T-MSCs), but not by primary cancer cells (PCCs) isolated from the same patients. Alongside IL-6, chemokines are important players in TME dynamics not only by directing the recruitment of different immune cell subsets to the tumors but also by eliciting responses from both tumor and stromal cells, and by regulating tumor angiogenesis, plasticity and survival [36]. Drugs capable of targeting production of IL-6 as well as at least a panel of relevant chemokines may therefore be worth exploring as a means to control the tumor promoting effect of the TME [28,37,38].

Recent *in vitro* experimental studies have suggested potentially potent anticancer properties of the lipid lowering drugs statins that include anti-inflammatory, immunomodulatory and antiangiogenic effects [39–43]. The inhibitory effects of simvastatin, a lipophilic statin, on tumor cell proliferation have been documented in a variety of cancers [44–51]. Simvastatin has been demonstrated to reduce tumor growth in inflammatory breast cancer models through the down-regulation of IL-6 [52] and to affect glioblastoma multiforme through TGF-β inhibition [53]. In lung cancer models, statins were observed to display a variety of effects that include induction of apoptosis [54], reduction of metastasis [55], and inhibition of angiogenesis [56] as well as tumor growth [57]. In addition, statins helped reduce lung cancer resistance to cis-platinum and tyrosine kinase inhibitors (TKIs) in the clinic [58–60]. However, studies and meta-analyses that investigated the relationship between statin administration and lung cancer patient survival [61–66] yielded controversial and inconsistent results. Thus the potential improvement in overall survival among lung cancer patients treated with statins and conventional anti-cancer therapy, suggested by observational studies, was not supported by evidence obtained from a small number of randomized controlled trials (RCTs) [63,67–69].

In the present work, using MSCs and PCCs from patients with NSCLC, we identified a cell type-specific pro-inflammatory signature composed of CCL2 (MCP-1) and IL-6 secretion as well as TLR4 expression by T-MSCs and CCL3 (MIP-1α) production by PCCs. On the basis of these observations and the tumor- and inflammation-inhibitory effects that statins are reported to exhibit, we tested their effects on primary MSC-PCC crosstalk in our model, using simvastatin. Our study shows potent effects of simvastatin on mesenchymal stromal cells isolated from the tumor microenvironment of patients as well as on PCCs resulting in reduction of signals that promote tumor growth.

2. Materials and methods

2.1. Isolation and characterization of MSCs and tumor cells

2.1.1. Mesenchymal stromal cells

Primary fresh tumor tissues and macroscopically normal adjacent tissues were obtained from 3 SCC patients (Patient 2, 3 and 4, according to previously used nomenclature [19]) following surgical resection at the Centre Universitaire Hospitalier Vaudois (CHUV). Patients 2, 3 and 4 were aged 64, 70, and 83, respectively, at the time of surgery. Patient 2 was female, and patients 3 and 4 were male. Pathologic tumor staging was performed at the CHUV and varied among the patients from T2a to T3. Patient-signed informed consent was obtained according to the guidelines of the Ethics Committee of Canton de Vaud (project authorization n° 131/12) and conforming to standards indicated by the Declaration of Helsinki. Normal-tissue-derived MSCs (N-MSCs) and T-MSCs were obtained after mechanical and enzymatic tissue disruption in IMDM (Gibco) supplemented with Collagenase II and IV (0,5 mg/ml, Gibco) and DNAse (0,1 mg/ml, Roche) for 2 h at 37 °C and passed through a 100 μm cell strainer. The resulting single cell bulk was cultured one night in MSC medium: IMDM + GlutaMAX (Gibco) supplemented with 10% fetal bovine serum (FBS) (PAN Biotech), 1% penicillin streptomycin (PS, Gibco), 1% non-essential amino acids (NEAA, Gibco) and 10 ng/ml platelet derived growth factor (PDGF, Prospec).

The following day, the culture medium was replaced with fresh medium and only adherent cells were kept. Upon reaching 80% confluence, cells were split 1:4–1:6 using trypsin-EDTA 0.25 mg/ml (Lonza, USA) and kept in culture in MSC medium. The MSCs phenotype was analyzed by flow cytometry and the differentiation potential into adipocytes, osteocytes and chondrocytes was assessed (detailed procedures in Ref. [26]). Cells between passage 2 and 9 were used in all experiments.

2.1.2. Tumor cells

Primary cancer cells (PCCs) from patients 2, 3 and 4 were cultured as single cells and allowed to form spheroids. The single cell-derived spheroids were cultured in ultra-low attachment flasks (Corning, Falcon) in KO medium: IMDM + GlutaMAX completed with 20% knockout serum (Gibco), 20 ng/ml leukemia inhibitory factor (LIF) (Prospec), 20 ng/ml recombinant human (rh) epidermal growth factor (EGF) (Prospec), 20 ng/ml fibroblast growth factor (FGF) (Prospec).

2.2. MSC-tumor cell co-culture

MSCs and tumor cells were co-cultured in transwell conditions at MSC:tumor cell ratio 1:3. Co-cultures were analyzed after 3 days. MSCs (50.000 cells/well) were seeded onto six-well plates (Costar, Corning incorporated). Tumor cells were seeded into 1,0 μm-pore insert of PET-membrane (Corning, Falcon) (150.000 cells/well). Controls were T- and N-MSCs or PCCs cultured alone. For all experiments, cells were cultured in MSC medium. At the end of co-culture or treatment, MSCs and PCCs were harvested, snap frozen and stored at −80 °C until RNA extraction.

2.2.1. Antibody and recombinant mediator treatment

MSCs (50.000 cells/well) were seeded in six-well plates, with or without tumor cells in transwell condition and treated for 3 days with anti-IL-6 2 μg/ml (MAB206 R&D), anti-TGF-β1/2/3 0.5–1 μg/ml (MAB1835 R&D), or anti-CCL2 4 μg/ml (MAB279 (R&D) antibodies in MSC medium. PCCs (150.000 cells/well), were cultured alone or with MSCs in transwell condition with anti-IL-6 2 μg/ml (MAB206 R&D), anti-TGF-β1/2/3 0.5–1 μg/ml (MAB1835 R&D), anti-CCL3 5 μg/ml (MAB670 (R&D). Untreated cells served as controls.

For recombinant mediator treatment, MSCs (50.000 cells/well) were treated for 3 days with human recombinant CCL3 10 ng/ml (270-LD-10; R&D). PCCs (150.000 cells/well) were treated for 3 days with human recombinant CCL2 10 ng/ml (279-MC-010; R&D), human IL-6 5 ng/ml (407652 Sigma) or TGF-β1 10–20 ng/ml (130-095-067 Miltenyi Biotec).

2.2.2. Simvastatin treatment

MSCs and PCCs were treated for 3 days with simvastatin 5 or 10 μM (S6196; Sigma) or for 24 h after 3 days in culture in their own culture medium. Stock solution was prepared in ethanol (initial concentration 100 mM). Cells treated with the same amount of ethanol were used as controls.

2.2.3. LPS treatment

For MSC stimulation with LPS, MSCs were cultured alone or with PCCs for 3 days to allow the cells to reach optimal cell density, then LPS 50 ng/ml (no. L2880; Sigma) was added for 24 h. Stimulation was maintained for 24 h based on observations by others [18] and the notion that the pro- or anti-inflammatory effects of TLR stimulation are duration-dependent. Unstimulated MSCs were generated in parallel and used as controls.

2.3. Cell culture in 3D hydrogels and F-actin staining

MSCs and PCCs were cultured in 96-well imaging plates containing pre-casted synthetic PEG-based hydrogels featuring a surface density gradient (3DProSeedTM hydrogel plate, cat.no. ECT-PS1; Ectica Technologies AG). MSCs (20.000 cells/well) or PCCs (30.000 cells/well) were seeded as cell suspension on top of the gel surfaces in 200 μl of their own medium (described in 2.1) supplemented with growth factors, in the presence or not of simvastatin 10 μM (S6196; Sigma), or with rhTGF-β1 20 ng/ml (130-095-067 Miltenyi Biotec). Because of a higher MSC seeding density required in 3D, simvastatin was used at higher concentration than in 2D culture. Culture medium was changed every 3 days, with addition of fresh growth factors and simvastatin/rhTGF-β1. At day 8, cells were fixed with 4% paraformaldehyde for 20 min at room temperature (RT), followed by two washes in PBS. Permeabilization was performed for 20 min at RT with 0.2% Triton X-100 in PBS, followed by two washes with PBS. Blocking was performed with 1%BSA/0.2% TX-100/5% goat serum in PBS for 45 min, at RT. Cells were stained for F-actin with Phalloidin-Atto 564 (no. 94072, Sigma) for 20 min, in the dark and rinsed three times with PBS before acquisition.

2.4. Cytokine quantification

Quantification of IL-6 and CCL2 secretion by MSCs were performed by ELISA (Human IL-6 DuoSet ELISA; no. DY206-05; R&D and Human CCL2/MCP-1 DuoSet ELISA; no. DY279; R&D) on MSC-tumor cell co-culture supernatants, according to the manufacturer's instructions. Samples were diluted 4 to 5-fold and analyzed in duplicates.

Quantification of CCL3 secretion by PCCs was performed by ELISA (Human CCL3/MIP-1α DuoSet ELISA; no. DY270; R&D) on MSC-tumor cell co-culture supernatants, according to the manufacturer's instructions. Samples were diluted 3-fold and analyzed in duplicates.

BDNF, Eotaxin/CCL11, EGF, FGF-2, GM-CSF, GRO alpha/CXCL1, HGF, NGF beta, LIF, IFN alpha, IFN gamma, IL-1 beta, IL-1 alpha, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10/CXCL10, MCP-1/CCL2, MIP-1 alpha/CCL3, MIP-1 beta/CCL4, RANTES/CCL5, SDF-1 alpha/CXCL12, TNF alpha, TNF beta/LTA, PDGF-BB, PLGF, SCF, VEGF-A and VEGF-D were assessed in the co-culture supernatants by Luminex assay (45-Plex Human ProcartaPlex, thermofischer).

2.5. Senescence assay for adherent cells

Senescence-associated (SA) expression of beta-galactosidase activity was histochemically detected using a senescence detection kit (ab65351; Abcam), according to the manufacturer's instructions. Adherent MSCs were cultured for 3 days in control medium or with simvastatin 5 μM before staining. Images were taken before staining, and 1, 24 and 96 h after staining.

2.6. Flow cytometry

2.6.1. Apoptosis

Cells were stained with APC Annexin V and the vital dye DAPI (10 μg/ml, Biotium) to identify early apoptotic cells, according to the manufacturer's instructions (FITC Annexin V Apoptosis Detection Kit; BD; no. 556547). Cells considered viable were both APC Annexin V and DAPI negative while cells that were in early apoptosis were APC Annexin V positive and DAPI negative. Cells that were in late apoptosis or already dead were both APC Annexin V and DAPI positive. All samples were acquired with Gallios Cytometer (Beckman Coulter) and data analyzed using FlowJo version10 software, following doublet exclusion.

2.6.2. TLR3/TLR4 expression

Cells were first washed in PBS (Bichsel AG, Interlaken CH) and stained 30 min at 4 °C with BB700-conjugated anti-CD284 (TLR4) antibody (no. 745946; BD; 1:10), FITC-conjugated anti-CD283 (TLR3) antibody (no. 130-100-001; Miltenyi; 1:10) and live/dead marker (1:200, Violet fluorescent reactive dye, Invitrogen) diluted in PBS and

finally fixed with PBS/PFA1%. Then, intracellular expression of TLR3/TLR4 was assessed with FITC-conjugated anti-CD283 (TLR3) antibody (no. 130-100-001; Miltenyi; 1:10) and BB700-conjugated anti-CD284 (TLR4) antibody (no. 745946; BD; 1:10) after permeabilization (0.2% BSA/0.1% Saponine in PBS, for 30 min at room temperature). TLR3/TLR4 expression was quantified within the live MSC population. Electronic compensation was set up using CompBeads (Beckman Coulter) to correct for fluorochrome spectral overlap. All samples were acquired with Gallios Cytometer (Beckman Coulter) and data analyzed using FlowJo version10 software following doublet and dead cell exclusion.

2.7. RNA extraction, cDNA synthesis and qRT-PCR

Total RNA was extracted using the RNeasy mini Kit (Qiagen), following the standard manufacturer protocol. For each sample, cDNA was synthesized by reverse transcription using M-MLV Reverse Transcriptase (Promega) according to manufacturer instructions. Levels of gene expression were determined using the 2-ΔΔCT methods [70] and samples analyzed in triplicates. Quantitative RT-PCR amplification was performed using SYBR® Green mix (Applied Biosystems). SYBR® Green primer sequences for the quantification of *IL6, TLR3, TLR4, CCL2, CCL3, CCL4, CCL5, PTGS2, IL8, VCAM1, ICAM1, TGFB1, CD274, IL1B, HLA-E, HMOX1* are listed in the table on Suppl. Exp. Procedures section. *GAPDH* or *TBP* (SYBR® Green) were used as housekeeping genes.

2.8. Microscopy

Images were taken with a Nikon Eclipse E800, digital camera DXM1200, at 40x or 100x magnification and at a resolution of 1280 × 1,024, and analyzed with the ACT-1 (v.2) software.

For 3D culture in hydrogels, images were taken with a spinning-disk confocal microscope Nikon Ti2 | Yokogawa CSU-W1 through a series of z-stacks (2.5 µm each for a 700 µm depth in total). Image visualization, 3D reconstruction and analysis were performed using Imaris software.

2.9. Quantification and statistical analysis

Statistical tests and graphics were generated by Prism version 7.03 (GraphPad Software Inc.). For qRT-PCR data, multiple t tests corrected for multiple comparisons using the Holm-Sidak method were used to compare mean expression levels between N- and T-MSCs for each patient. Multiple group analysis was performed by one-way ANOVA test followed by Dunnett's multiple test comparisons. P values < 0.05 were considered statistically significant and are denoted by asterisks:

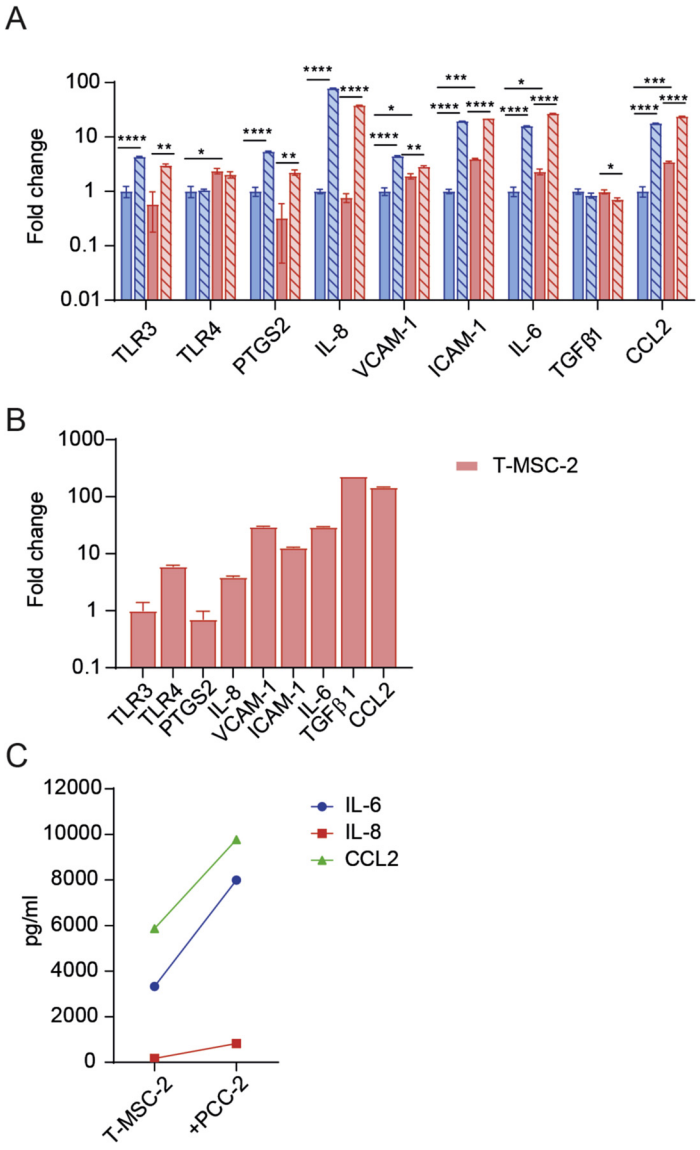


Fig. 1. MSC and PCC Gene Expression and Secretory Profiles. (A) Relative gene expression of *TLR3, TLR4, PTGS2, IL8, VCAM1, ICAM1, IL6, TGFB1* and *CCL2* by N- and T-MSCs from Patient 2 (MSC-2) alone and in co-culture with PCCs from the same patient, as assessed by qPCR and shown as a fold change in expression, plotted on a logarithmic scale. Results show the mean ± SD of triplicates, normalized to *TBP* expression. For each gene, N-MSC-2 expression is set as the control condition (=1). Statistical significance was determined by multiple t-tests using the Holm-Sidak Method, with alpha = 0.05 (* indicates significance at $p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$). (B) Expression by T-MSC-2 of relevant genes relative to the *TLR3* expression level (set as the control condition). (C) Panel of highly secreted factors (pg/ml) by T-MSC-2 alone and in the presence of PCC-2.

∗p < 0.05; ∗∗p < 0.01; ∗∗∗p < 0.001; ∗∗∗∗p < 0.0001; ns, not significant. Error bars represent the SD or SEM.

3. Results

3.1. Tumor-derived mesenchymal stromal cells harbor a pro-inflammatory phenotype

Stromal cells were isolated from dissociated primary lung squamous-cell carcinoma (SCC) as well as from adjacent tumor-free tissue from three patients and verified for functional and phenotypic MSC features as defined by the International Society for Cellular Therapy (ISCT) [71]. Accordingly, both tumor and tumor-free lung-tissue-derived stromal cells that we selected for our study were adherent to plastic under standard culture conditions, expressed comparable levels of CD105, CD73, and CD90, lacked expression of lineage markers and underwent differentiation to osteocytes, adipocytes, and chondrocytes *in vitro* in response to appropriate stimulation (see detailed protocol in Ref. [19]). We previously demonstrated that normal-tissue-derived MSCs (N-MSCs) differ from T-MSCs in the relative expression of a repertoire of genes implicated in immunomodulation and inflammation, particularly *IL6* and *TLR4* [19,26]. In addition, we isolated primary cancer cells (PCCs) from the same patients and co-cultured them with MSCs to address their relationship and reciprocal modulation.

In the present study, we co-cultured patient-derived N-MSCs and PCCs in transwell conditions and observed that PCCs induce up-regulation of *IL6, CCL2, VCAM1* and *ICAM1* in N-MSCs (Fig. 1A), which, along with *TGFB1* are among the most highly expressed genes in T-

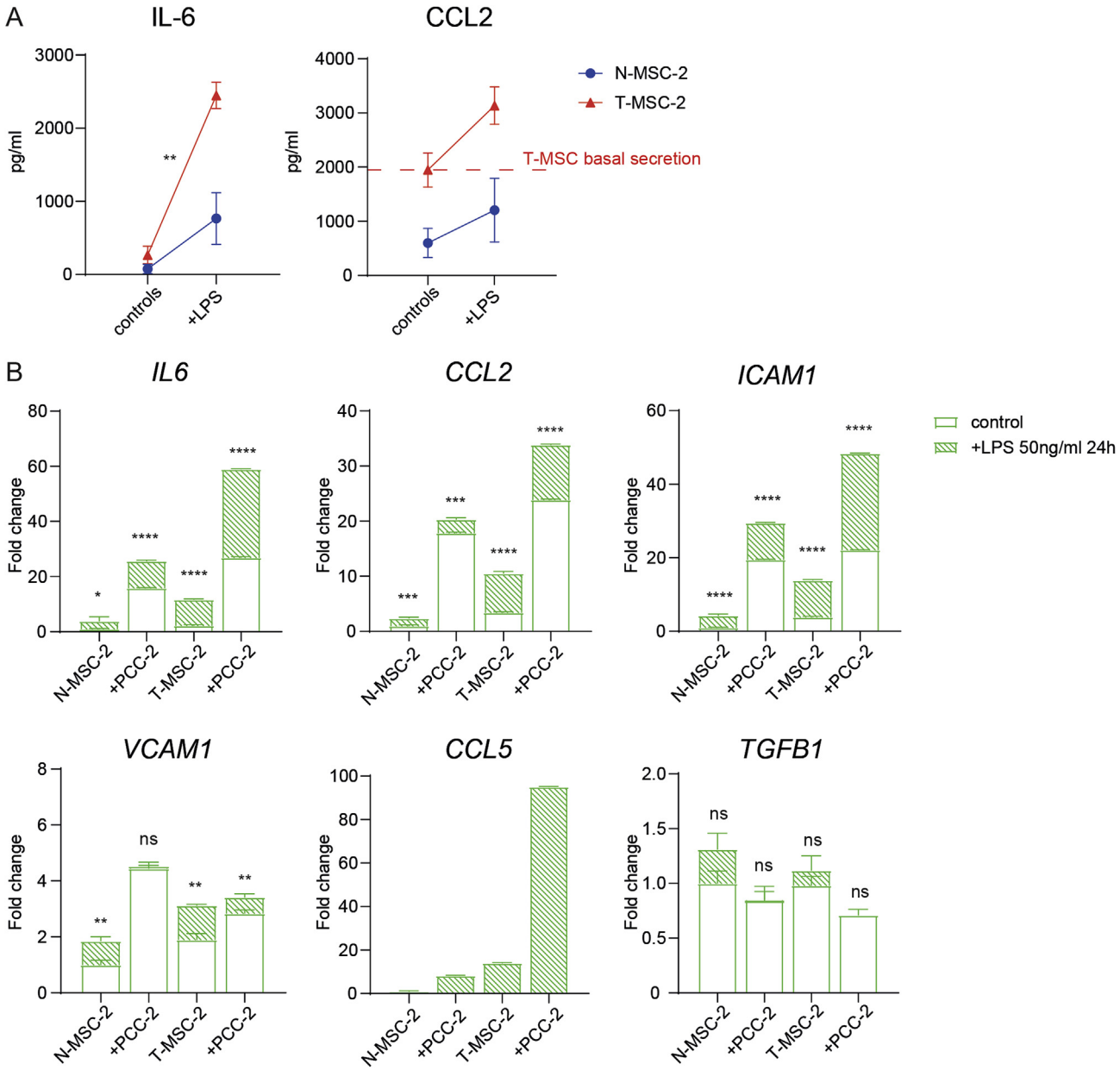


Fig. 2. LPS Stimulation of MSCs. (A) IL-6 and CCL2 secretion (pg/ml) by N- and T-MSC-2. Data are representative of two separate experiments, each in duplicate. Results show the mean ± SD computed using the four datasets. Statistical significance was determined by multiple t-tests using the Holm-Sidak Method. (B) Relative gene expression of *IL6, CCL2, ICAM1, VCAM1, CCL5* and *TGFB1* by MSCs alone or co-cultured with PCCs, after 24 h-stimulation with LPS (50 ng/ml) compared to control conditions. Gene expression modulation by LPS was statistically determined for each condition (N- or T-MSCs alone or with PCCs from Patient 2), using multiple t-tests with the Holm-Sidak Method for statistical significance, with alpha = 0.05 (* indicates significance at p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001).

MSCs in the absence of PCC co-culture (Fig. 1B). Broad screening of the T-MSC secretory profile by Luminex uncovered pronounced secretion of several pro-inflammatory cytokines and chemokines, including IL-6 and CCL2 (Figs. 1C and S1A (i)) whose secretion was significantly increased in the presence of PCCs (Fig. 1C). Contrary to other studies [72–81], both mediators were found to be secreted exclusively by MSCs and were undetectable in PCC cultured alone (Fig. S1A (ii)).

Toll-like receptor 4 was identified as one of the proteins whose expression correlated with the tumor-related signature of MSCs [26]. Moreover, MSCs are referred to as MSC1 or MSC2 according to the predominance of TLR4 or TLR3 expression, which corresponds to a pro-inflammatory or immunosuppressive phenotype, respectively [17,82]. In the present study we observed that lung cancer-derived T-MSCs display intracellular expression of both TLR3 and 4 (Figs. S1B and S1C), the latter being higher than the former. *TLR3*, but not *TLR4* expression was induced by the presence of PCCs (Fig. S1B). Consistent with reports showing that TLR4 agonists induce CCL2 and IL-6 secretion in MSCs [83–85], we observed increased IL-6 and CCL2 expression and secretion by MSCs treated with lipopolysaccharide (LPS) (Fig. 2A and B). As both mediators are strongly induced in MSCs cultured in the presence of PCCs, LPS had only a minor incremental effect on their production when added to the co-cultures (Fig. S1D). In addition, LPS significantly increased MSC expression *of ICAM1, VCAM1* and induced *de novo* expression of *CCL5* but did not affect *TGFB1* expression (Fig. 2B). The effect on MSCs of LPS stimulation was therefore similar to that exerted by PCCs, except for *CCL5* whose expression required LPS stimulation.

Constitutive and PCC- as well as LPS-inducible secretion of IL-6 and CCL2 and expression of *ICAM1* and *VCAM1* by T-MSCs suggest their implication in promoting inflammation, immune cell recruitment and cell-cell interactions in the TME of early-stage lung cancer.

3.2. Simvastatin affects cell adhesion and attenuates the pro-inflammatory features of MSCs

To attenuate the expression of several key mediators of inflammation by MSCs and tumor cells using a single drug, we sought to target cell metabolism. Statins provide an attractive option to this end because in addition to their lipid-lowering properties, they display pleiotropic effects on cell metabolism, which may explain, at least in part, their observed inhibition of inflammation and tumor growth [43,49,86–90]. We therefore addressed the effect of simvastatin on the pro-inflammatory profile of T-MSCs cultured alone and in the presence of PCCs. Our first observation was that under statin treatment, MSCs lost cell-to-cell contact and tended to detach from the plate (Fig. 3A), suggesting an effect on the expression and/or function of cell-matrix and cell-cell adhesion receptors. MSCs cultured in 3D-hydrogels displayed marked changes in morphology and cell-cell communication in response to simvastatin, suggesting disruption of physiological cell adhesion mechanisms (Fig. 3B). Consistent with this notion, we observed downregulation of the cell-cell adhesion receptor gene *VCAM1* (Fig. 3C). In contrast, simvastatin induced apoptosis only in a tiny fraction of MSCs, as assessed by flow cytometry (Fig. 3D), without causing senescence (Fig. 3E) in MSCs.

In addition to *VCAM1,* statin treatment reduced *TLR3, IL6,* and *CCL2* expression (Fig. 4A and E), whereas it increased that of *TGFB1, TLR4* and *PTGS2* in T-MSCs cultured alone and in the presence of PCCs (Fig. 4A and E). IL-6 was more strongly affected in T- than in N-MSCs treated with the drug (Figs. 4B and S2AFigs. 4B and S2A), providing direct evidence that simvastatin may be effective in restoring inflammatory mediator production to physiological levels. Indeed, simvastatin treatment of T-MSCs, cultured alone or with PCCs, reduced their IL-6 secretion to the levels displayed by N-MSCs cultured under the same conditions (Fig. 4B) but, in contrast, increased IL-8 secretion (Fig. 4C). Thus, exposure to statin attenuated important constituents of the pro-inflammatory signature of T-MSCs, as reflected by inhibition of IL-6 and CCL2 secretion (Fig. 4B–D) and *VCAM1* expression, particularly in the context of PCC co-culture (Fig. 4A and E). By contrast, it promoted *PTGS2, TGFB1* and *HMOX1* expression, which reflects an immunosuppressive phenotype (Fig. 4E).

3.3. Lung carcinoma cells secrete CCL3

PCCs displayed elevated secretion of the chemokine CCL3, but no production of CCL2 and IL-6 (Fig. 5A (i)). CCL3 is reported to be secreted by both cancer and stromal cells, including BM-MSCs [91] and to contribute to tumorigenesis [92]. However, MSCs isolated from lung carcinoma did not produce CCL3 (Fig. 5A (i)). Previous studies had shown that inflammatory cytokines, including IL-6 can induce CCL3 secretion [93,94], which in turn stimulates CCL5 and IL-6 production [92], supporting tumour progression. In contrast, we observed that co-culture with T-MSCs, which are robust providers of IL-6, decreased CCL3 secretion by PCCs (Fig. 5A (ii)), suggesting that other T-MSC-derived soluble mediators may override the previously described stimulatory effect of IL-6 on CCL3 production by tumor cells and possibly substitute for its pro-tumorigenic effects. Discrepancies between our observations and other studies may be explained by the origin of MSCs and PCCs. Our present work provides the opportunity to probe the behavior of primary cells, isolated directly from patients with lung squamous cell carcinoma, in contrast to the vast majority of studies, which have been conducted using bone marrow-derived MSCs and cancer cell lines.

3.4. Simvastatin may be effective at targeting PCCs isolated at early stages of lung cancer

Simvastatin treatment of PCCs induced changes in cell morphology and affected spheroid formation. In control conditions (no treatment), PCCs formed smooth well-defined spheroids (Fig. 5B (i)). When PCCs cultured as single cells (at culture initiation) were treated with the drug, their ability to form regular spheroids was impaired (Fig. 5B (ii)). The structures they formed had an irregular shape and a large fraction of the cells underwent apoptosis (Fig. 5C). However, established PCC spheroids were less sensitive to simvastatin (Fig. 5B (iv)), suggesting that simvastatin may have an effect at early stages of tumor development - or may even provide a preventive measure – rather than in advanced or metastatic disease. Consistent with this notion, simvastatin induced apoptosis in PCCs in culture prior to spheroid formation (Annexin-DAPI staining) (Fig. 5C) irrespective of the presence of MSCs (Fig. S2B). The observed PCC apoptosis could therefore not be explained by simvastatin-mediated inhibition of IL-6 production by MSCs (Figs. S2B and S2C). Simvastatin-treated PCCs displayed decreased CCL3 gene expression and secretion irrespective of the presence or absence of T-MSCs (Fig. 5D and E).

3.5. IL-6, CCL2 and CCL3 interplay

To explore the interplay between IL-6, CCL2 and CCL3, we used inhibitors of each mediator on the one hand, and stimulation with recombinant proteins on the other (Fig. 6A). CCL2 had no effect on PCC CCL3 production, as stimulation of PCCs by rhCCL2 affected neither their constitutive CCL3 secretion (Fig. 6C (i)) nor its restoration following its suppression by statin treatment (Fig. 6C (i)). Similarly, inhibition of IL-6 secreted by T-MSCs in PCC-MSC co-culture failed to decrease PCC CCL3 secretion (Fig. 6C (ii)).

Although simvastatin inhibited secretion of both IL-6 and CCL2 by MSCs (Fig. 4A and E), neutralization of CCL2 in MSC-PCC co-culture did not decrease IL-6 secretion but on the contrary resulted in its slight increase, which may constitute a compensatory mechanism in response to the inhibition (Fig. 6B (ii)). Neutralization of CCL3 secreted by PCCs co-cultured with MSCs, slightly increased MSC IL-6 secretion (Fig. 6B (ii)) but decreased CCL2 secretion (Fig. 6B (ii)). Blocking IL-6 secretion by T-MSCs also decreased their CCL2 production (Fig. 6B (i)), whereas

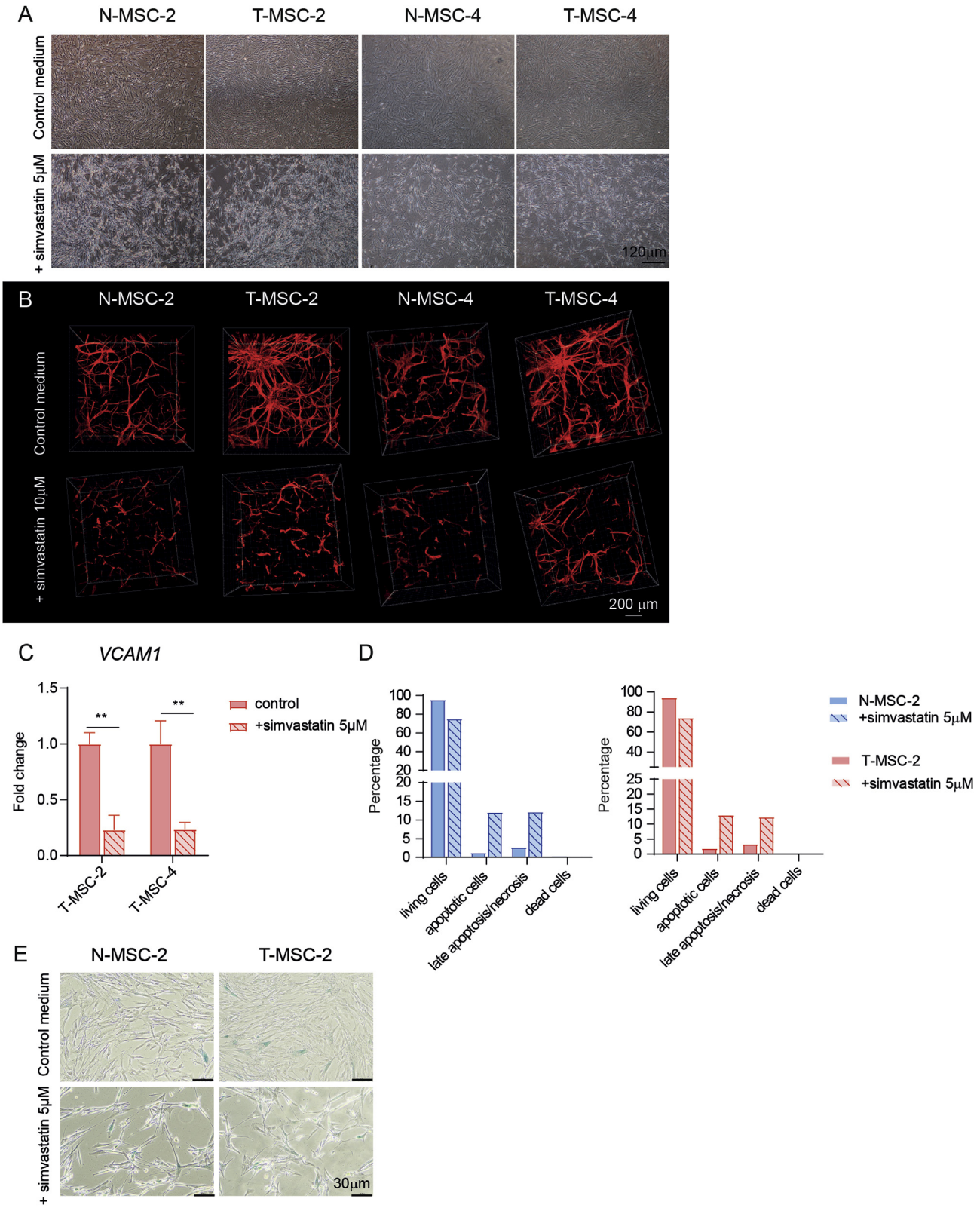


Fig. 3. Mesenchymal Stromal Cells and Statin Treatment. (A) Images of MSCs in 2-D culture from patients 2 and 4, after 3 days in culture with or without simvastatin at 5 µM. Scale bar = 120 µm. (B) MSCs (stained with Phalloidin-Atto 564) from Patients 2 and 4 cultured for 8 days in a 3-D-hydrogel gradient plate in control medium or with 10 µM simvastatin. 3-D reconstruction of 2.5 µm z-stacks is shown. Scale bar = 200 µm. (C) *VCAM1* gene expression by T-MSCs from Patient 2 and 4 cultured with or without simvastatin at 5 µM, as assessed by qPCR and shown as a fold change in expression. For each patient, T-MSC expression set as the control condition (=1), was normalized to *TBP* (MSC-2) or *GAPDH* (MSC-4) expression. Statistical significance was determined by multiple t-tests with the Holm-Sidak Method, with alpha = 0.05 (* indicates significance at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). (D) Percentages of living, apoptotic, late apoptotic/necrotic and dead MSC-2, cultured with or without statin at 5 µM. The cell phenotype was assessed by flow cytometry. (E) Senescent MSC-2 detected by β-galactosidase activity (blue staining), after 24 h of X-gal staining. X-gal staining was performed on MSCs cultured for 3 days with or without statin 5 µM treatment. Scale bar = 30 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

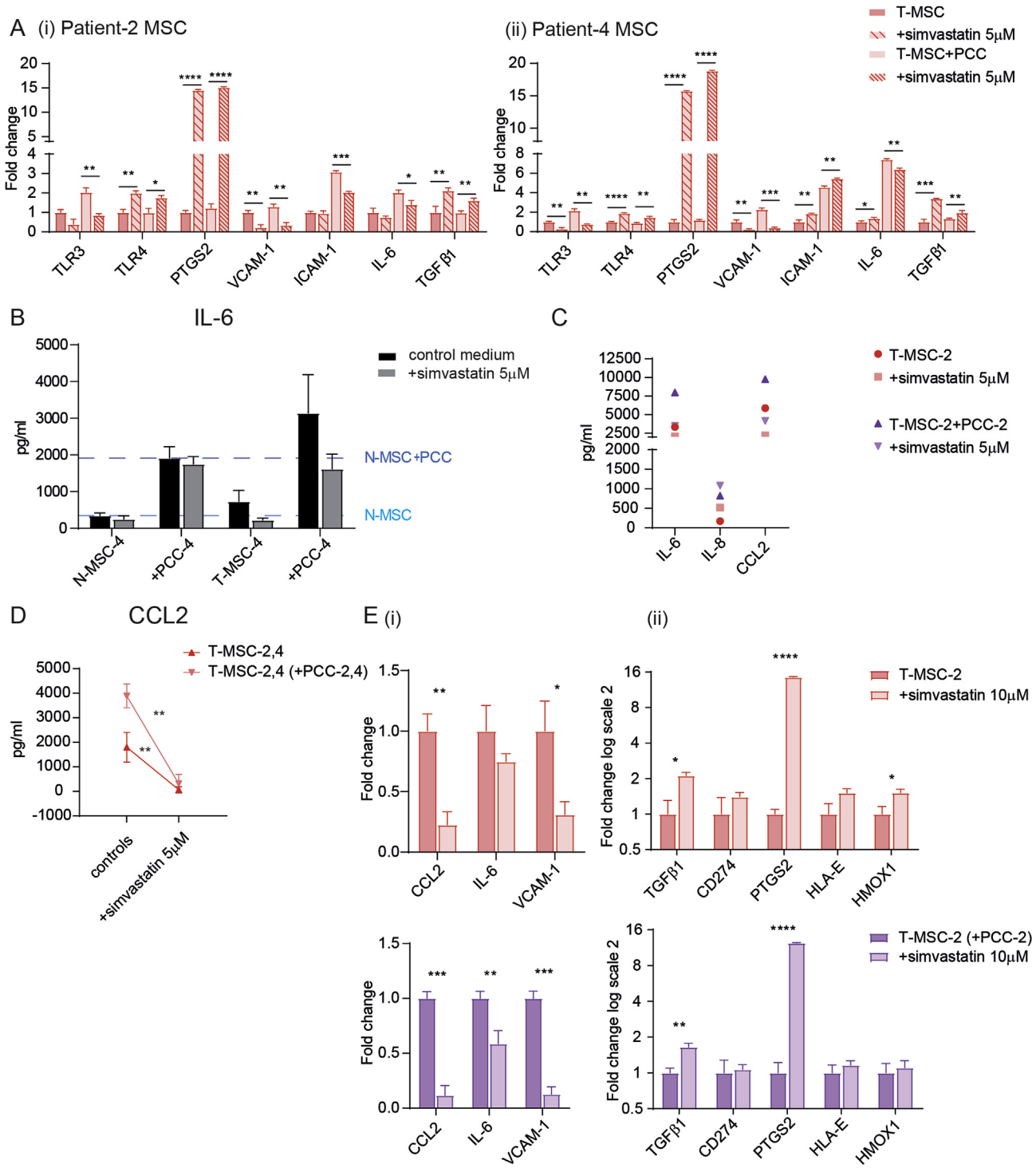
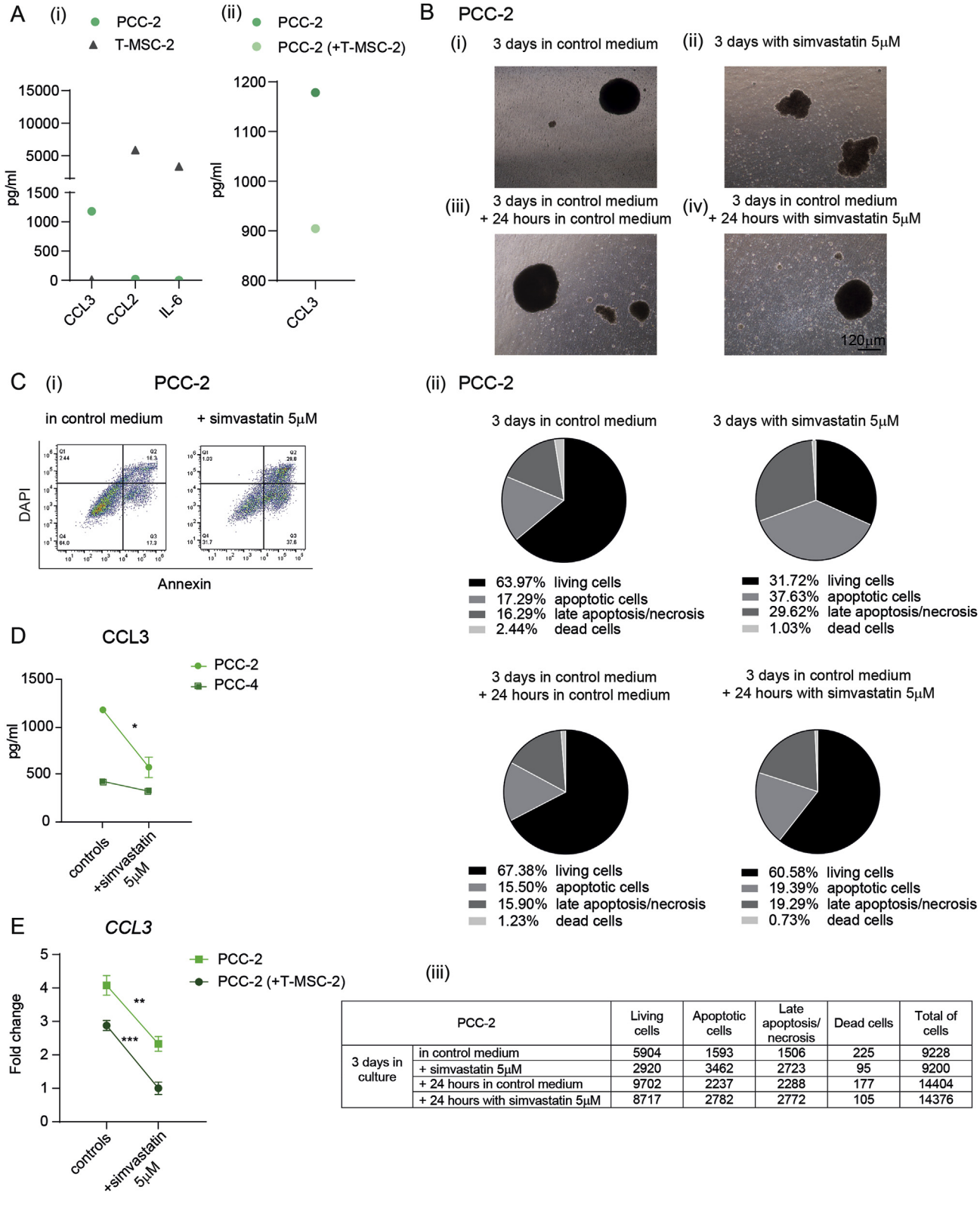


Fig. 4. Simvastatin treatment modulates T-MSC expression of adhesion receptors and soluble mediators of inflammation. (A) Relative gene expression of *TLR3, TLR4, PTGS2, VCAM1, ICAM1, IL6* and *TGFB1* by T-MSC-2 (i) and T-MSC-4 (ii), cultured alone or with PCCs, with or without simvastatin 5 μM, as assessed by qPCR and expressed as a fold change in expression. Expression of each gene in resting T-MSCs was set as the control (=1). Results show the mean ± SD of triplicates, normalized to *TBP* (MSC-2) or *GAPDH* (MSC-4) expression. (B) IL-6 secretion (pg/mL) by MSCs under statin treatment or in control medium, after 3 days in culture alone or with PCCs. Results show the mean ± SD of 3 experiments with MSC-4, in duplicate. Blue lines depict N-MSC secretion of IL-6, cultured alone or with PCCs. (C) IL-6, IL-8 and CCL2 secretion by T-MSC-2 alone or with PCC-2, under statin 5 μM treatment or in control medium. Results of one experiment, in duplicate are shown. (D) CCL2 secretion (pg/ml) by T-MSCs with or without PCC co-culture, under statin treatment and in control medium. Results show the mean ± SD of 3 experiments, each in duplicate (MSC-2, MSC-4). (E) Relative gene expression by T-MSC-2, treated or untreated with simvastatin for 3 days, alone (red) or in the presence of PCCs (purple). (i) Panel of pro-inflammatory genes: *CCL2, IL6, VCAM1*; (ii) Panel of immunosuppressive genes: *TGFB1, CD274, PTGS2, HLA-E, HMOX1*. Results show the mean ± SD of triplicate assays. For (A), (D) and (E), statistical significance was determined by multiple t-tests with the Holm-Sidak Method, with alpha = 0.05 (* indicates significance at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



(iii)

PCC-2		Living cells	Apoptotic cells	Late apoptosis/ necrosis	Dead cells	Total of cells
3 days in culture	in control medium	5904	1593	1506	225	9228
	+ simvastatin 5µM	2920	3462	2723	95	9200
	+ 24 hours in control medium	9702	2237	2288	177	14404
	+ 24 hours with simvastatin 5µM	8717	2782	2772	105	14376

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recombinant CCL3 induced secretion of both CCL2 and IL-6 in MSCs (Fig. 6B (i)).

Because simvastatin treatment increased TGF-β expression (Fig. 4A and E), we addressed the effect of recombinant TGF-β on our cells. Similar to statins, TGF-β inhibited PCC growth (Fig. 6E (i)) and down-regulated their production of CCL3 (Fig. 6C (ii)) in the presence of T-MSCs, as well as MSC production of CCL2 (Fig. 6B and D). However, contrary to statins, TGF-β induced IL-6 expression and secretion and increased stromal cell adhesion (Fig. 6B and E (ii)). TGF-β has notoriously pleiotropic effects on cancer cells, which depend, in part on the stage of cancer progression. In normal and premalignant cells, TGF-β suppresses tumor progression directly through cell-autonomous tumor-

Fig. 5. Primary Cancer Cells and Statin Treatment. (A) Secretion of CCL3, CCL2 and IL-6 by PCC-2 and T-MSC-2 (pg/ml) cultured alone (i) or in co-culture (ii). (B) Formation of spheroids, as a characteristic of PCCs, after three-day culture, with (ii) or without simvastatin treatment (5 μM) (i). PCC spheroids were dissociated into single cells and simvastatin was administered at culture initiation for 3 days (ii) or after spheroid formation, at day 3, for 24 h (iv). PCC-2 cultured for 3 (i) or 4 (iii) days in standard medium provided the control conditions. (C) (i) Representative flow density dot plots showing expression of Annexin and DAPI by PCC-2, in control medium and under simvastatin treatment (5 μM) for 3 days. Grey-scale pie charts (ii) represent the percentages of living, apoptotic, late apoptotic/necrotic and dead PCCs, cultured in control medium or with statin 5 μM for 3 days or with statin for 24 h. Cells were analyzed by flow cytometry, using Annexin and DAPI staining; (iii) Table of the total number of events (cell number) observed for the different culture conditions and cell states (living, apoptotic, late apoptotic/necrotic and dead PCC-2). (D) CCL3 secretion (pg/ml) by PCC-2 and PCC-4 in control medium or after statin 5 μM treatment for 3 days. Data are representative of three different experiments, each in duplicate. Statistical significance was determined by two-tailed unpaired *t*-test (* indicates significance at $p \leq 0.05$). (E) Relative *CCL3* gene expression by PCC-2 alone or cultured with T-MSC-2, in control medium or with statin 5 μM. *CCL3* expression by PCCs cultured in the presence of T-MSCs with statin was set as the control condition (=1). Statistical significance was determined by multiple t-tests with the Holm-Sidak Method, with alpha = 0.05 (* indicates significance at $p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

suppressive effects, including cytostasis, differentiation and apoptosis, as well as indirectly via effects on the stroma that include suppression of inflammation and stromal cell-derived mitogens [95]. However, when cancer cells lose the tumor suppressive responses to TGF-β, they can use TGF-β to modulate the microenvironment to elude immune surveillance and to induce the production of pro-tumorigenic cytokines. We observed that in co-culture TGF-β inhibited MSC and PCC production of CCL2 and CCL3, respectively, two chemokines implicated in monocyte recruitment, and promoted a more adherent phenotype in MSCs, suggesting a potentially more differentiated state, such as that of cancer-associated fibroblasts (CAFs).

In summary, simvastatin down-regulated CCL3 in PCCs whereas CCL3 up-regulated CCL2 and IL-6 in MSCs. Simvastatin may therefore downregulate CCL2 and IL-6 expression in MSC both directly and by inhibition of PCC production of CCL3 (Fig. 6F).

4. Discussion

Mesenchymal stromal cells display a high degree of phenotypic and functional plasticity that provides them with the ability to help resolve or maintain diverse pathophysiological situations. They participate in maintaining tissue homeostasis by sensing the degree of an inflammatory response and adapting their functions to enhance or attenuate inflammation and contribute to tissue repair [82]. However, they also harbor potent immunosuppressive properties [96] and, as part of the TME, can guide tumor cell fate and support tumor progression. Despite their pro-tumorigenic potential, their natural tumor tropism, which provides a means for drug delivery and consequently targeted treatment, has led clinical studies to suggest that MSCs may hold promise for cancer therapy [97–102]. In the present work, we sought to determine how MSCs adapt and contribute to chronic inflammation in the TME of human lung SCC. We observed that primary SCC cells secrete CCL3 and CCL4 and enhance the pro-inflammatory properties of MSCs by inducing them to produce IL-6 and CCL2, which in turn creates a tumor-permissive microenvironment. We then found that simvastatin can disrupt the tumor cell-MSC crosstalk by affecting both MSCs and PCCs but in distinct ways. Simvastatin attenuated the adhesion and pro-inflammatory signature of MSCs, reducing their secretion of IL-6 and CCL2 while enhancing their immunosuppressive features. Its effects on PCC included inhibition of CCL3 secretion along with induction of apoptosis in a significant fraction of cells. Our observations propose mechanisms implicated in maintaining the TME that emerge from MSC-PCC crosstalk and the potential repurposing of the commonly used drug simvastatin toward attenuating cancer-sustaining inflammation.

4.1. Pro-inflammatory lung tumor microenvironment

The TME consists not only of a heterogeneous population of cancer cells but also a variety of resident and infiltrating host cells, including MSCs, secreted factors and extracellular matrix glycoproteins, glycolipids and glycosaminoglycans. It is in a constant state of turnover whose dynamics are largely dictated by the inflammation triggered and maintained by tumor cells. The inflammatory response itself is shaped by the properties of the tumor cells, which may highjack some of its key mediators to promote their own survival and resistance to adverse conditions such as hypoxia and anti-cancer therapy. Tumor progression is therefore profoundly influenced by interactions between cancer cells and their environment, which ultimately drive the growth dynamics of the primary tumor and determine its invasion of adjacent tissues as well as the establishment of dormant micrometastases and their exit from dormancy [28,103,104].

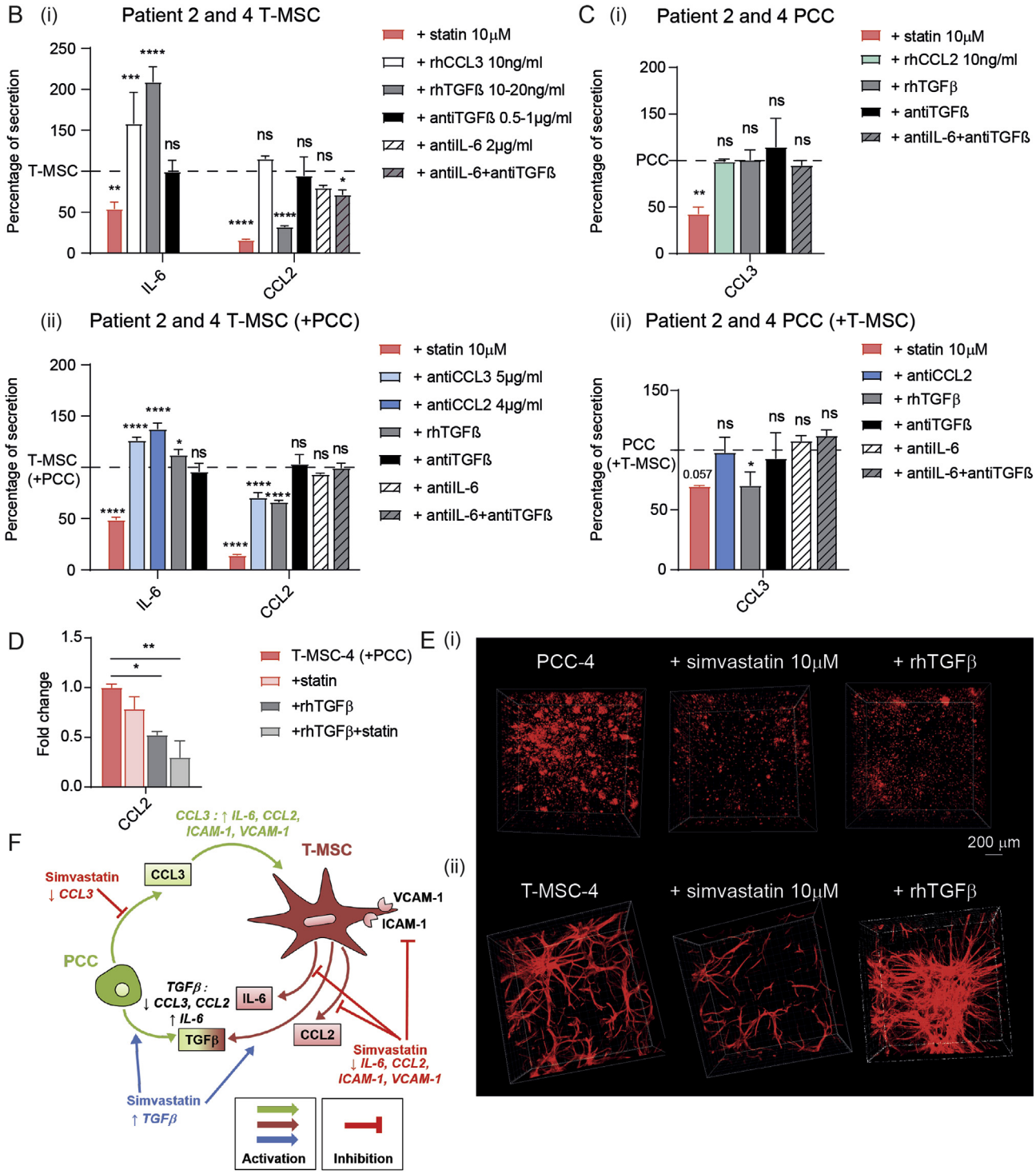
The cytokines and chemokines on whose action tumor cells rely for survival, plasticity, migration and invasion may be secreted by the tumor cells themselves and/or by the immune and stromal cells that compose the TME. In the present study, we identified IL-6, CCL2, CCL3 and TGF-β as four highly secreted soluble mediators in the TME of human lung SCC, with IL-6 and CCL2 being secreted by T-MSCs, CCL3, by PCCs and TGF-β by both cell types.

The pleiotropic effects of IL-6 and TGF-β have been extensively studied in cancer and converge toward the promotion of tumor progression [31,32,34]. However, the effect of chemokines on tumor growth is somewhat less well explored. Whereas chemokines were identified and characterized based on their ability to direct chemotaxis of immune cell subsets [36,105,106], mounting evidence indicates that many can directly target non-immune cells — including tumor cells and MSCs — in the TME, and regulate tumor cell proliferation, plasticity, invasiveness and metastasis. Chemokines affect tumor immunity by shaping the composition of the immune cell infiltrate in addition to influencing cancer cell behavior that includes response to therapy [36]. Insight from recent investigation of the cancer chemokine network is revealing parallels between the pathogenesis of inflammation and malignancy and uncovering aspects of their mutual relationship that may offer new therapeutic perspectives [106].

CCL2 is secreted by a wide range of cells including epithelial and stromal cells, and promotes tumor cell proliferation, plasticity, survival, extravasation and metastasis as well as tumor vascularization [72–81]. CCL2 recruits monocytes to injured or dysfunctional tissue [107] and has been shown to regulate VCAM-1 and ICAM-1 expression, consistent with requirements for monocyte-endothelial cell adhesion [108]. Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, TGF-β), which induce CCL2 production by endothelial and stromal cells can also increase CCL2 production by tumor cells [81]. We observed upregulation of CCL2 secretion by MSCs in the presence of lung cancer cells or upon LPS treatment, consistent with observations showing that TLR4 agonists induce CCL2 as well as IL-6 secretion in MSCs [84,85]. CCL2 is suggested to participate in lung cancer cell resistance to chemotherapy *in vivo* [109] and high levels of CCL2 have been associated with poor prognosis in lung adenocarcinoma [110] but not in SCC. Although *in vitro* studies and humanized animal models indicate that the presence of CCL2 favors cancer progression [111], a clinicopathological study of 65 patients with advanced NSCLC suggested that the expression of CCL2 in tumor tissue correlates with longer survival [112]. However, a more recent study explored stromal cell heterogeneity in the lung TME at single cell resolution and identified several stromal cell subpopulations, one of which displayed elevated expression of CCL2 and was associated with poor survival in NSCLC patients [113].

A

	T-MSC	PCC	T-MSC+PCC
Secreted cytokines	CCL2, IL-6, TGFβ	CCL3, TGFβ	CCL2, IL-6, CCL3, TGFβ
Used inhibitors	α-CCL2, α-IL-6, α-TGFβ	α-CCL3, α-TGFβ	α-CCL2, α-IL-6, α-CCL3, α-TGFβ
Used stimulators	rhCCL3, rhTGFβ	rhCCL2, rhTGFβ	



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CCL3, which was strongly secreted by PCCs from SCC, has been demonstrated to contribute to tumorigenesis [92,114] and suggested to be secreted by both cancer and stromal cells, including BM-MSCs [91]. However, our observations suggest that MSCs isolated from lung SCC do not produce CCL3. Previous studies have shown that inflammatory cytokines, including TNF-α, IL-1β and IL-6 can induce CCL3 secretion [93,94], which in turn stimulates CCL5, IL-6, and MMP2/9 production [92], supporting tumor progression. Our observations indicate that CCL3 promotes MSC secretion of IL-6 and CCL2 but that alone it is not sufficient to reproduce the effect of PCC co-culture on IL-6 and CCL2

Fig. 6. MSC-PCC crosstalk: role of CCL3, CCL2 and IL-6. (A) Summary table of the cytokines secreted by MSCs and PCCs and of the different culture conditions used. (B) IL-6 and CCL2 secretion (pg/ml) by T-MSCs alone (i) or T-MSCs co-cultured with PCCs from Patients 2 and 4 (ii) under the indicated culture conditions: simvastatin treatment; neutralization of CCL2, CCL3, IL-6 or TGF-β; stimulation with recombinant (rh) CCL3 or TGF-β. Secretion was normalized to that of resting T-MSCs or T-MSCs co-cultured with PCCs in control medium (=100%, in dashed line). (C) CCL3 secretion (pg/ml) by PCC alone (i) or with T-MSC (ii) under the indicated culture conditions: control medium; CCL2 or TGF-β agonist stimulation to mimic MSC secretion in PCC monoculture; IL-6 or TGF-β inhibition, alone or in combination; CCL2 inhibition in PCC-MSC co-culture. Data in (B) and (C) represent the mean values ± SD of three experiments, in duplicate. (D) Relative gene expression of *CCL2* by T-MSC-4 alone, treated by statin, stimulated with TGF-β or both. Results show the mean ± SEM of one experiment, in triplicate. Statistical significance for (B), (C) and (D) was determined by one-way ANOVA with Dunnett correction for multiple comparisons, with alpha = 0.05 (* indicates significance at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). (E) PCCs (i) and MSCs (ii) (stained with Phalloidin-Atto 564) from Patient 4 cultured for 8 days in a 3-D-hydrogel gradient plate in different culture conditions: in control medium, under 10 μM simvastatin treatment or with 20 ng/ml rhTGF-β stimulation. 3-D reconstruction of 2.5 μm z-stacks is shown. Scale bar = 200 μm. (F) Summary diagram of MSC-PCC crosstalk and of the effects of simvastatin on secreted factors.

secretion by MSCs, suggesting the contribution of additional tumor cell-derived mediators to the observed MSC IL-6 and CCL2 production.

4.2. Statins in lung cancers

Resolution of cancer-promoting inflammation as an approach for anticancer therapy has paved the way toward broadening the use of anti-inflammatory agents such as NSAIDs and lipid-lowering drugs such as statins. Statins are widely used to reduce hypercholesterolemia and manage cardio- and cerebrovascular diseases. They inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme in the mevalonate pathway. Mevalonate is a precursor of metabolites that regulate the small GTPases Ras and Rho, which in turn regulate signal transduction of membrane receptors crucial for the transcription of genes involved in cell proliferation, differentiation, angiogenesis and survival and for the regulation of migration mechanics [51,87,88].

Several recent observations have highlighted the anticancer properties of statins. One study suggested that statins might reduce lung cancer-associated death, whether they are administered prior to or after diagnosis [62]. In phase II trials, two studies recorded improved survival among lung cancer patients who received simvastatin plus gefinitib compared to gefinitib alone [66,67] and both suggested that statin administration had beneficial effects on lung cancer patient survival. Mechanistic studies have shown reduced proliferation and migration and increased apoptosis in several types of cancer cells, including those derived from lung adenocarcinoma in response to statins [115,116]. Other studies have reported that simvastatin augments apoptosis in NSCLC cell lines [54,116,117]. Like other statins, simvastatin serves as an inhibitor of HMG-CoA reductase by interfering with the metabolism of the mevalonate pathway. However, its anticancer activity is suggested to be, at least in part, HMG-CoA-independent [86] and to affect cell survival pathways. Thus, in lung cancer, simvastatin is reported to upregulate expression of the pro-apoptotic Bcl-2 gene family member BIM to promote apoptosis [118]. Others have suggested that simvastatin enhances oxidative stress and upregulates expression of superoxide dismutase 2, inhibiting lung cancer cell proliferation [119]. Others still have provided evidence that pyroptosis may be implicated in the anticancer effects of simvastatin [117]. All these observations have been made using cell lines, whereas the present study identifies a pro-apoptotic effect of simvastatin on primary cancer cells, isolated from patients with lung SCC and known to be more resistant to anti-tumor therapies. In addition to promotion of apoptosis, statins decrease CCL3 secretion by PCCs. The observed reduction of CCL3 production was not attributed to cell death, because the degree of CCL3 inhibition was considerably higher than could be explained by the fraction of cells displaying markers of apoptosis. In a model of multiple myeloma, statins were observed to inhibit LPS-induced CCL3 expression and secretion by tumor cells via inhibition of Ras/ERK and Ras/Akt pathways [120].

4.3. Simvastatin and MSCs

Our present work demonstrates that simvastatin affects not only tumor cells, but also the stromal compartment, with, in addition to attenuation of IL-6 and CCL2 secretion, markedly decreased adhesion of MSCs to substrate and strong downregulation of ICAM-1 and VCAM-1 expression (more markedly so in T-MSCs than in N-MSCs). Simvastatin also affects the phenotype of T-MSCs, by switching the highly pro-inflammatory profile of T-MSCs to a more immunosuppressive one, characterized by high TGF-β, HMOX-1 and PGE2 expression.

As immunotherapy becomes an increasingly central to the therapeutic arsenal against lung cancer, targeting inflammation and affecting the balance between inflammation and immunity will require thorough understanding of the changes induced in the TME and their effect on responses to immunotherapy. We observed a potent effect of statin treatment on MSC IL-6 secretion that was counterbalanced by an increase in their immunosuppressive profile, with high PGE2 and TGF-β, which may affect the immune landscape, favoring an immunosuppressive microenvironment. In this sense, statins may be an interesting therapeutic option to explore in graft-versus-host disease, where the immunosuppressive function of MSCs has already been addressed. Earlier, we demonstrated the role of T-MSC-derived prostaglandin E2 on NK cell inhibition of IFN-γ secretion and downregulation of NK activating receptors upon direct interaction [19]. The use of statins may affect directly the proliferation and survival of tumor cells and alter the ability of stromal cells present in the TME to sustain tumor growth and inhibit immune responses. Statins have been shown to downregulate perforin-mediated NK cell cytotoxicity, but not perforin-independent pathways of cytotoxicity such as FasL/Fas [121]. One of the next challenges to address will be to assess the effect of simvastatin on MSC-NK crosstalk, especially IFN-γ secretion.

Taken together, our observations reveal mechanisms by which interactions with PCCs shape the MSC profile to maintain chronic inflammation and immune dysregulation. We identify simvastatin as a promising anti-inflammatory and anti-tumor drug that targets both the tumor stroma and tumor cells and disrupts the MSC-PCC crosstalk.

CRediT authorship contribution statement

Sabine Galland: Conceptualization, Investigation, Formal analysis, Methodology, Writing - review & editing, Funding acquisition. **Patricia Martin:** Investigation. **Giulia Fregni:** Resources, Writing - review & editing. **Igor Letovanec:** Resources. **Ivan Stamenkovic:** Conceptualization, Formal analysis, Methodology, Resources, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2020.05.005.

References

[1] H.F. Dvorak, Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing, N. Engl. J. Med. 315 (1986) 1650–1659, https://doi.org/10.1056/NEJM198612253152606.
[2] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867, https://doi.org/10.1038/nature01322.
[3] F. Balkwill, A. Mantovani, Inflammation and cancer: back to Virchow? Lancet 357 (2001) 539–545, https://doi.org/10.1016/S0140-6736(00)04046-0.
[4] A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Cancer-related inflammation, Nature 454 (2008) 436–444, https://doi.org/10.1038/nature07205.
[5] M. Schäfer, S. Werner, Cancer as an overhealing wound: an old hypothesis revisited, Nat. Rev. Mol. Cell Biol. 9 (2008) 628–638, https://doi.org/10.1038/nrm2455.
[6] J. Candido, T. Hagemann, Cancer-related inflammation, J. Clin. Immunol. 33 (Suppl 1) (2013) S79–S84, https://doi.org/10.1007/s10875-012-9847-0.
[7] F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability, Carcinogenesis 30 (2009) 1073–1081, https://doi.org/10.1093/carcin/bgp127.
[8] J.A. Aguirre-Ghiso, Models, mechanisms and clinical evidence for cancer dormancy, Nat. Rev. Canc. 7 (2007) 834–846, https://doi.org/10.1038/nrc2256.
[9] J.A. Aguirre-Ghiso, M.S. Sosa, Emerging topics on disseminated cancer cell dormancy and the paradigm of metastasis, Annu. Rev. Cell Biol. 2 (2018) 377–393, https://doi.org/10.1146/annurev-cancerbio-030617-050446.
[10] D.F. Quail, J.A. Joyce, Microenvironmental regulation of tumor progression and metastasis, Nat. Med. 19 (2013) 1423–1437, https://doi.org/10.1038/nm.3394.
[11] K.M. Bussard, L. Mutkus, K. Stumpf, C. Gomez-Manzano, F.C. Marini, Tumor-associated stromal cells as key contributors to the tumor microenvironment, Breast Cancer Res. 18 (2016), https://doi.org/10.1186/s13058-016-0740-2.
[12] L. Wan, K. Pantel, Y. Kang, Tumor metastasis: moving new biological insights into the clinic, Nat. Med. 19 (2013) 1450–1464, https://doi.org/10.1038/nm.3391.
[13] C. Giallongo, D. Tibullo, G. Camiolo, N.L. Parrinello, A. Romano, F. Puglisi, A. Barbato, C. Conticello, G. Lupo, C.D. Anfuso, G. Lazzarino, G. Li Volti, G.A. Palumbo, F. Di Raimondo, TLR4 signaling drives mesenchymal stromal cells commitment to promote tumor microenvironment transformation in multiple myeloma, Cell Death Dis. 10 (2019) 704, https://doi.org/10.1038/s41419-019-1959-5.
[14] M.E. Bernardo, W.E. Fibbe, Mesenchymal stromal cells: sensors and switchers of inflammation, Cell Stem Cell 13 (2013) 392–402, https://doi.org/10.1016/j.stem.2013.09.006.
[15] C.A. Dumitru, H. Hemeda, M. Jakob, S. Lang, S. Brandau, Stimulation of mesenchymal stromal cells (MSCs) via TLR3 reveals a novel mechanism of autocrine priming, Faseb. J. 28 (2014) 3856–3866, https://doi.org/10.1096/fj.14-250159.
[16] M. Krampera, Mesenchymal stromal cell "licensing": a multistep process, Leukemia 25 (2011) 1408–1414, https://doi.org/10.1038/leu.2011.108.
[17] R.S. Waterman, S.L. Tomchuck, S.L. Henkle, A.M. Betancourt, A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype, PloS One 5 (2010), https://doi.org/10.1371/journal.pone.0010088.
[18] R.M. Petri, A. Hackel, K. Hahnel, C.A. Dumitru, K. Bruderek, S.B. Flohe, A. Paschen, S. Lang, S. Brandau, Activated tissue-resident mesenchymal stromal cells regulate natural killer cell immune and tissue-regenerative function, Stem Cell Reports 9 (2017) 985–998, https://doi.org/10.1016/j.stemcr.2017.06.020.
[19] S. Galland, J. Vuille, P. Martin, I. Letovanec, A. Caignard, G. Fregni, I. Stamenkovic, Tumor-derived mesenchymal stem cells use distinct mechanisms to block the activity of natural killer cell subsets, Cell Rep. 20 (2017) 2891–2905, https://doi.org/10.1016/j.celrep.2017.08.089.
[20] A.H. Klopp, A. Gupta, E. Spaeth, M. Andreeff, F. Marini, Concise review: dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? Stem Cell. 29 (2011) 11–19, https://doi.org/10.1002/stem.559.
[21] S.M. Ridge, F.J. Sullivan, S.A. Glynn, Mesenchymal stem cells: key players in cancer progression, Mol. Canc. 16 (2017) 31, https://doi.org/10.1186/s12943-017-0597-8.
[22] J. Stagg, Mesenchymal stem cells in cancer, Stem Cell Rev. 4 (2008) 119–124, https://doi.org/10.1007/s12015-008-9030-4.
[23] M.W. Lee, S. Ryu, D.S. Kim, J.W. Lee, K.W. Sung, H.H. Koo, K.H. Yoo, Mesenchymal stem cells in suppression or progression of hematologic malignancy: current status and challenges, Leukemia 33 (2019) 597–611, https://doi.org/10.1038/s41375-018-0373-9.
[24] F. Papaccio, F. Paino, T. Regad, G. Papaccio, V. Desiderio, V. Tirino, Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development, Stem Cells Transl Med 6 (2017) 2115–2125, https://doi.org/10.1002/sctm.17-0138.
[25] S. Gottschling, M. Granzow, R. Kuner, A. Jauch, E. Herpel, E.C. Xu, T. Muley, P.A. Schnabel, F.J.F. Herth, M. Meister, Mesenchymal stem cells in non-small cell lung cancer–different from others? Insights from comparative molecular and functional analyses, Lung Canc. 80 (2013) 19–29, https://doi.org/10.1016/j.lungcan.2012.12.015.
[26] G. Fregni, M. Quinodoz, E. Möller, J. Vuille, S. Galland, C. Fusco, P. Martin, I. Letovanec, P. Provero, C. Rivolta, N. Riggi, I. Stamenkovic, Reciprocal modulation of mesenchymal stem cells and tumor cells promotes lung cancer metastasis, EBioMedicine 29 (2018) 128–145, https://doi.org/10.1016/j.ebiom.2018.02.017.
[27] M. Gazdic, B. Simovic Markovic, N. Jovicic, M. Misirkic-Marjanovic, V. Djonov, V. Jakovljevic, N. Arsenijevic, M.L. Lukic, V. Volarevic, Mesenchymal stem cells promote metastasis of lung cancer cells by downregulating systemic antitumor immune response, Stem Cell. Int. (2017) 6294717, https://doi.org/10.1155/2017/6294717 2017.
[28] N.K. Altorki, G.J. Markowitz, D. Gao, J.L. Port, A. Saxena, B. Stiles, T. McGraw, V. Mittal, The lung microenvironment: an important regulator of tumour growth and metastasis, Nat. Rev. Canc. 19 (2019) 9–31, https://doi.org/10.1038/s41568-018-0081-9.
[29] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.
[30] H. Lemjabbar-Alaoui, O.U. Hassan, Y.-W. Yang, P. Buchanan, Lung cancer: biology and treatment options, Biochim. Biophys. Acta 1856 (2015) 189–210, https://doi.org/10.1016/j.bbcan.2015.08.002.
[31] T. Tanaka, M. Narazaki, T. Kishimoto, IL-6 in inflammation, immunity, and disease, Cold Spring Harb Perspect Biol 6 (2014), https://doi.org/10.1101/cshperspect.a016295.
[32] N. Kumari, B.S. Dwarakanath, A. Das, A.N. Bhatt, Role of interleukin-6 in cancer progression and therapeutic resistance, Tumour Biol 37 (2016) 11553–11572, https://doi.org/10.1007/s13277-016-5098-7.
[33] J. Chen, T. Ji, D. Wu, S. Jiang, J. Zhao, H. Lin, X. Cai, Human mesenchymal stem cells promote tumor growth via MAPK pathway and metastasis by epithelial mesenchymal transition and integrin α5 in hepatocellular carcinoma, Cell Death Dis. 10 (2019) 1–12, https://doi.org/10.1038/s41419-019-1622-1.
[34] D.T. Fisher, M.M. Appenheimer, S.S. Evans, The two faces of IL-6 in the tumor microenvironment, Semin. Immunol. 26 (2014) 38–47, https://doi.org/10.1016/j.smim.2014.01.008.
[35] S.A. Jones, B.J. Jenkins, Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer, Nat. Rev. Immunol. 18 (2018) 773–789, https://doi.org/10.1038/s41577-018-0066-7.
[36] N. Nagarsheth, M.S. Wicha, W. Zou, Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy, Nat. Rev. Immunol. 17 (2017) 559–572, https://doi.org/10.1038/nri.2017.49.
[37] S. Kang, T. Tanaka, M. Narazaki, T. Kishimoto, Targeting interleukin-6 signaling in clinic, Immunity 50 (2019) 1007–1023, https://doi.org/10.1016/j.immuni.2019.03.026.
[38] S.M. Crusz, F.R. Balkwill, Inflammation and cancer: advances and new agents, Nat. Rev. Clin. Oncol. 12 (2015) 584–596, https://doi.org/10.1038/nrclinonc.2015.105.
[39] Y. Ahmadi, A. Ghorbanihaghjo, H. Argani, The balance between induction and inhibition of mevalonate pathway regulates cancer suppression by statins: a review of molecular mechanisms, Chem. Biol. Interact. 273 (2017) 273–285, https://doi.org/10.1016/j.cbi.2017.06.026.
[40] R. Zeiser, Immune modulatory effects of statins, Immunology 154 (2018) 69–75, https://doi.org/10.1111/imm.12902.
[41] A. Fatehi Hassanabad, Current perspectives on statins as potential anti-cancer therapeutics: clinical outcomes and underlying molecular mechanisms, Transl. Lung Cancer Res. 8 (2019) 692–699, https://doi.org/10.21037/tlcr.2019.09.08.
[42] A. Fatehi Hassanabad, S.A. McBride, Statins as potential therapeutics for lung cancer: molecular mechanisms and clinical outcomes, Am. J. Clin. Oncol. 42 (2019) 732–736, https://doi.org/10.1097/COC.0000000000000579.
[43] A. Chimento, I. Casaburi, P. Avena, F. Trotta, A. De Luca, V. Rago, V. Pezzi, R. Sirianni, Cholesterol and its metabolites in tumor growth: therapeutic potential of statins in cancer treatment, Front. Endocrinol. 9 (2019), https://doi.org/10.3389/fendo.2018.00807.
[44] I. Takeda, S.-I. Maruya, T. Shirasaki, H. Mizukami, T. Takahata, J.N. Myers, S. Kakehata, S. Yagihashi, H. Shinkawa, Simvastatin inactivates beta1-integrin and extracellular signal-related kinase signaling and inhibits cell proliferation in head and neck squamous cell carcinoma cells, Canc. Sci. 98 (2007) 890–899, https://doi.org/10.1111/j.1349-7006.2007.00471.x.
[45] M. Tsubaki, T. Takeda, T. Kino, N. Obata, T. Itoh, M. Imano, K. Mashimo, D. Fujiwara, K. Sakaguchi, T. Satou, S. Nishida, Statins improve survival by inhibiting spontaneous metastasis and tumor growth in a mouse melanoma model, Am J Cancer Res 5 (2015) 3186–3197.
[46] Y.-C. Yang, W.-F. Huang, L.-M. Chuan, D.-W. Xiao, Y.-L. Zeng, D.-A. Zhou, G.-Q. Xu, W. Liu, B. Huang, Q. Hu, In vitro and in vivo study of cell growth inhibition of simvastatin on chronic myelogenous leukemia cells, CHEManager 54 (2008) 438–446, https://doi.org/10.1159/000158663.
[47] C. Spampanato, S. De Maria, M. Sarnataro, E. Giordano, M. Zanfardino, S. Baiano, M. Cartenì, F. Morelli, Simvastatin inhibits cancer cell growth by inducing apoptosis correlated to activation of Bax and down-regulation of BCL-2 gene expression, Int. J. Oncol. 40 (2012) 935–941, https://doi.org/10.3892/ijo.2011.1273.
[48] N.W.C.J. van de Donk, M.M.J. Kamphuis, H.M. Lokhorst, A.C. Bloem, The cholesterol lowering drug lovastatin induces cell death in myeloma plasma cells,

Leukemia 16 (2002) 1362–1371, https://doi.org/10.1038/sj.leu.2402501.
[49] L. Matusewicz, J. Meissner, M. Toporkiewicz, A.F. Sikorski, The effect of statins on cancer cells–review, Tumour Biol 36 (2015) 4889–4904, https://doi.org/10.1007/s13277-015-3551-7.
[50] M. Yanae, M. Tsubaki, T. Satou, T. Itoh, M. Imano, Y. Yamazoe, S. Nishida, Statin-induced apoptosis via the suppression of ERK1/2 and Akt activation by inhibition of the geranylgeranyl-pyrophosphate biosynthesis in glioblastoma, J. Exp. Clin. Canc. Res. 30 (2011) 74, https://doi.org/10.1186/1756-9966-30-74.
[51] Y. Kobayashi, K. Banno, H. Kunitomi, S. Nagai, T. Takahashi, M. Anko, M. Iijima, T. Takeda, Y. Matoba, K. Nakamura, K. Tsuji, E. Tominaga, D. Aoki, Is anti-dyslipidemic statin use for cancer prevention a promising drug repositioning approach? Eur. J. Canc. Prev. 28 (2019) 562–567, https://doi.org/10.1097/CEJ.0000000000000497.
[52] A.R. Wolfe, N.J. Trenton, B.G. Debeb, R. Larson, B. Ruffell, K. Chu, W. Hittelman, M. Diehl, J.M. Reuben, N.T. Ueno, W.A. Woodward, Mesenchymal stem cells and macrophages interact through IL-6 to promote inflammatory breast cancer in pre-clinical models, Oncotarget 7 (2016) 82482–82492, https://doi.org/10.18632/oncotarget.12694.
[53] A. Xiao, B. Brenneman, D. Floyd, L. Comeau, K. Spurio, I. Olmez, J. Lee, I. Nakano, J. Godlewski, A. Bronisz, N. Kagaya, K. Shin-ya, B. Purow, Statins affect human glioblastoma and other cancers through TGF-β inhibition, Oncotarget 10 (2019) 1716–1728, https://doi.org/10.18632/oncotarget.26733.
[54] K.-E. Hwang, K.-S. Na, D.-S. Park, K.-H. Choi, B.-R. Kim, H. Shim, E.-T. Jeong, H.-R. Kim, Apoptotic induction by simvastatin in human lung cancer A549 cells via Akt signaling dependent down-regulation of survivin, Invest. N. Drugs 29 (2011) 945–952, https://doi.org/10.1007/s10637-010-9450-2.
[55] H. Liu, Z. Wang, Y. Li, W. Li, Y. Chen, Simvastatin prevents proliferation and bone metastases of lung adenocarcinoma in vitro and in vivo, Neoplasma 60 (2013) 240–246, https://doi.org/10.4149/neo_2013_032.
[56] J. Chen, B. Liu, J. Yuan, J. Yang, J. Zhang, Y. An, L. Tie, Y. Pan, X. Li, Atorvastatin reduces vascular endothelial growth factor (VEGF) expression in human non-small cell lung carcinomas (NSCLCs) via inhibition of reactive oxygen species (ROS) production, Mol Oncol 6 (2012) 62–72, https://doi.org/10.1016/j.molonc.2011.11.003.
[57] J. Hanai, N. Doro, A.T. Sasaki, S. Kobayashi, L.C. Cantley, P. Seth, V.P. Sukhatme, Inhibition of lung cancer growth: ATP citrate lyase knockdown and statin treatment leads to dual blockade of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/AKT pathways, J. Cell. Physiol. 227 (2012) 1709–1720, https://doi.org/10.1002/jcp.22895.
[58] J. Chen, T. Lan, J. Hou, J. Zhang, Y. An, L. Tie, Y. Pan, J. Liu, X. Li, Atorvastatin sensitizes human non-small cell lung carcinomas to carboplatin via suppression of AKT activation and upregulation of TIMP-1, Int. J. Biochem. Cell Biol. 44 (2012) 759–769, https://doi.org/10.1016/j.biocel.2012.01.015.
[59] K.-E. Hwang, S.-J. Kwon, Y.-S. Kim, D.-S. Park, B.-R. Kim, K.-H. Yoon, E.-T. Jeong, H.-R. Kim, Effect of simvastatin on the resistance to EGFR tyrosine kinase inhibitors in a non-small cell lung cancer with the T790M mutation of EGFR, Exp. Cell Res. 323 (2014) 288–296, https://doi.org/10.1016/j.yexcr.2014.02.026.
[60] O. Fiala, M. Pesek, J. Finek, M. Minarik, L. Benesova, Z. Bortlicek, O. Topolcan, Statins augment efficacy of EGFR-TKIs in patients with advanced-stage non-small cell lung cancer harbouring KRAS mutation, Tumour Biol 36 (2015) 5801–5805, https://doi.org/10.1007/s13277-015-3249-x.
[61] S.F. Nielsen, B.G. Nordestgaard, S.E. Bojesen, Statin use and reduced cancer-related mortality, N. Engl. J. Med. 367 (2012) 1792–1802, https://doi.org/10.1056/NEJMoa1201735.
[62] C.R. Cardwell, Ú.M. Menamin, C.M. Hughes, L.J. Murray, Statin use and survival from lung cancer: a population-based cohort study, Cancer Epidemiol. Biomark. Prev. 24 (2015) 833–841, https://doi.org/10.1158/1055-9965.EPI-15-0052.
[63] M.J. Seckl, C.H. Ottensmeier, M. Cullen, P. Schmid, Y. Ngai, D. Muthukumar, J. Thompson, S. Harden, G. Middleton, K.M. Fife, B. Crosse, P. Taylor, S. Nash, A. Hackshaw, Multicenter, phase III, randomized, double-blind, placebo-controlled trial of pravastatin added to first-line standard chemotherapy in small-cell lung cancer (LUNGSTAR), J. Clin. Oncol. 35 (2017) 1506–1514, https://doi.org/10.1200/JCO.2016.69.7391.
[64] Y. Chen, X. Li, R. Zhang, Y. Xia, Z. Shao, Z. Mei, Effects of statin exposure and lung cancer survival: a meta-analysis of observational studies, Pharmacol. Res. 141 (2019) 357–365, https://doi.org/10.1016/j.phrs.2019.01.016.
[65] M.A.M. Farooqi, N. Malhotra, S.D. Mukherjee, S. Sanger, S.K. Dhesy-Thind, P. Ellis, D.P. Leong, Statin therapy in the treatment of active cancer: a systematic review and meta-analysis of randomized controlled trials, PloS One 13 (2018), https://doi.org/10.1371/journal.pone.0209486.
[66] J.J. Lin, N. Ezer, K. Sigel, G. Mhango, J.P. Wisnivesky, The effect of statins on survival in patients with stage IV lung cancer, Lung Canc. 99 (2016) 137–142, https://doi.org/10.1016/j.lungcan.2016.07.006.
[67] J.-Y. Han, S.-H. Lee, N.J. Yoo, L.S. Hyung, Y.J. Moon, T. Yun, H.T. Kim, J.S. Lee, A randomized phase II study of gefitinib plus simvastatin versus gefitinib alone in previously treated patients with advanced non–small cell lung cancer, Clin. Canc. Res. 17 (2011) 1553–1560, https://doi.org/10.1158/1078-0432.CCR-10-2525.
[68] Y. Lee, K.H. Lee, G.K. Lee, S.-H. Lee, K.Y. Lim, J. Joo, Y.J. Go, J.S. Lee, J.-Y. Han, Randomized phase II study of afatinib plus simvastatin versus afatinib alone in previously treated patients with advanced nonadenocarcinomatous non-small cell lung cancer, Cancer Res Treat 49 (2017) 1001–1011, https://doi.org/10.4143/crt.2016.546.
[69] D.-K. Xia, Z.-G. Hu, Y.-F. Tian, F.-J. Zeng, Statin use and prognosis of lung cancer: a systematic review and meta-analysis of observational studies and randomized controlled trials, Drug Des. Dev. Ther. 13 (2019) 405–422, https://doi.org/10.2147/DDDT.S187690.
[70] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[71] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317, https://doi.org/10.1080/14653240600855905.
[72] M.J. Wolf, A. Hoos, J. Bauer, S. Boettcher, M. Knust, A. Weber, N. Simonavicius, C. Schneider, M. Lang, M. Stürzl, R.S. Croner, A. Konrad, M.G. Manz, H. Moch, A. Aguzzi, G. van Loo, M. Pasparakis, M. Prinz, L. Borsig, M. Heikenwalder, Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway, Canc. Cell 22 (2012) 91–105, https://doi.org/10.1016/j.ccr.2012.05.023.
[73] V. Goede, L. Brogelli, M. Ziche, H.G. Augustin, Induction of inflammatory angiogenesis by monocyte chemoattractant protein-1, Int. J. Canc. 82 (1999) 765–770, https://doi.org/10.1002/(SICI)1097-0215(19990827)82:5<765::AID-IJC23>3.0.CO;2-F.
[74] H. Saji, M. Koike, T. Yamori, S. Saji, M. Seiki, K. Matsushima, M. Toi, Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma, Cancer 92 (2001) 1085–1091, https://doi.org/10.1002/1097-0142(20010901)92:5<1085::aid-cncr1424>3.0.co;2-k.
[75] L. Bonapace, M.-M. Coissieux, J. Wyckoff, K.D. Mertz, Z. Varga, T. Junt, M. Bentires-Alj, Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis, Nature 515 (2014) 130–133, https://doi.org/10.1038/nature13862.
[76] S.C. Robinson, K.A. Scott, F.R. Balkwill, Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNF-α, Eur. J. Immunol. 32 (2002) 404–412, https://doi.org/10.1002/1521-4141(200202)32:2<404::AID-IMMU404>3.0.CO;2-X.
[77] W.B. Fang, I. Jokar, A. Zou, D. Lambert, P. Dendukuri, N. Cheng, CCL2/CCR2 chemokine signaling coordinates survival and motility of breast cancer cells through Smad3 protein- and p42/44 mitogen-activated protein kinase (MAPK)-dependent mechanisms, J. Biol. Chem. 287 (2012) 36593–36608, https://doi.org/10.1074/jbc.M112.365999.
[78] A. Tsuyada, A. Chow, J. Wu, G. Somlo, P. Chu, S. Loera, T. Luu, A.X. Li, X. Wu, W. Ye, S. Chen, W. Zhou, Y. Yu, Y.-Z. Wang, X. Ren, H. Li, P. Scherle, Y. Kuroki, S.E. Wang, CCL2 mediates cross-talk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells, Canc. Res. 72 (2012) 2768–2779, https://doi.org/10.1158/0008-5472.CAN-11-3567.
[79] Z. Ma, X. Zhao, M. Deng, Z. Huang, J. Wang, Y. Wu, D. Cui, Y. Liu, R. Liu, G. Ouyang, Bone marrow mesenchymal stromal cell-derived periostin promotes B-all progression by modulating CCL2 in leukemia cells, Cell Rep. 26 (2019) 1533–1543, https://doi.org/10.1016/j.celrep.2019.01.034 e4.
[80] S.Y. Lim, A.E. Yuzhalin, A.N. Gordon-Weeks, R.J. Muschel, Targeting the CCL2-CCR2 signaling axis in cancer metastasis, Oncotarget 7 (2016) 28697–28710, https://doi.org/10.18632/oncotarget.7376.
[81] T. Yoshimura, The chemokine MCP-1 (CCL2) in the host interaction with cancer: a foe or ally? Cell. Mol. Immunol. 15 (2018) 335–345, https://doi.org/10.1038/cmi.2017.135.
[82] S. Galland, I. Stamenkovic, Mesenchymal stromal cells in cancer: a review of their immunomodulatory functions and dual effects on tumor progression, J. Pathol. (2019), https://doi.org/10.1002/path.5357.
[83] S.L. Tomchuck, K.J. Zwezdaryk, S.B. Coffelt, R.S. Waterman, E.S. Danka, A.B. Scandurro, Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses, Stem Cell. 26 (2008) 99–107, https://doi.org/10.1634/stemcells.2007-0563.
[84] H. Kang, K.-H. Kim, J. Lim, Y.-S. Kim, J. Heo, J. Choi, J. Jeong, Y. Kim, S.W. Kim, Y.-M. Oh, M.-S. Choo, J. Son, S.J. Kim, H.J. Yoo, W. Oh, S.J. Choi, S.W. Lee, D.-M. Shin, The therapeutic effects of human mesenchymal stem cells primed with sphingosine-1 phosphate on pulmonary artery hypertension, Stem Cell. Dev. 24 (2015) 1658–1671, https://doi.org/10.1089/scd.2014.0496.
[85] D.J. Kota, B. DiCarlo, R.A. Hetz, P. Smith, C.S. Cox, S.D. Olson, Differential MSC activation leads to distinct mononuclear leukocyte binding mechanisms, Sci. Rep. 4 (2014) 4565, https://doi.org/10.1038/srep04565.
[86] K.K.W. Chan, A.M. Oza, L.L. Siu, The statins as anticancer agents, Clin. Canc. Res. 9 (2003) 10–19.
[87] K. Hindler, C.S. Cleeland, E. Rivera, C.D. Collard, The role of statins in cancer therapy, Oncol. 11 (2006) 306–315, https://doi.org/10.1634/theoncologist.11-3-306.
[88] P.J. Mullen, R. Yu, J. Longo, M.C. Archer, L.Z. Penn, The interplay between cell signalling and the mevalonate pathway in cancer, Nat. Rev. Canc. 16 (2016) 718–731, https://doi.org/10.1038/nrc.2016.76.
[89] O. Bedi, V. Dhawan, P.L. Sharma, P. Kumar, Pleiotropic effects of statins: new therapeutic targets in drug design, Naunyn-Schmiedeberg's Arch. Pharmacol. 389 (2016) 695–712, https://doi.org/10.1007/s00210-016-1252-4.
[90] P. Quist-Paulsen, Statins and inflammation: an update, Curr. Opin. Cardiol. 25 (2010) 399–405, https://doi.org/10.1097/HCO.0b013e3283398e53.
[91] G. Nishikawa, K. Kawada, J. Nakagawa, K. Toda, R. Ogawa, S. Inamoto, R. Mizuno, Y. Itatani, Y. Sakai, Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression via CCR5, Cell Death Dis. 10 (2019) 264, https://doi.org/10.1038/s41419-019-1508-2.
[92] J.M. da Silva, T.P. Moreira dos Santos, L.M. Sobral, C.M. Queiroz-Junior, M.A. Rachid, A.E.I. Proudfoot, G.P. Garlet, A.C. Batista, M.M. Teixeira, A.M. Leopoldino, R.C. Russo, T.A. Silva, Relevance of CCL3/CCR5 axis in oral

carcinogenesis, Oncotarget 8 (2017) 51024–51036, https://doi.org/10.18632/oncotarget.16882.
[93] R.C. Russo, A.L. Alessandri, C.C. Garcia, B.F. Cordeiro, V. Pinho, G.D. Cassali, A.E.I. Proudfoot, M.M. Teixeira, Therapeutic effects of evasin-1, a chemokine binding protein, in bleomycin-induced pulmonary fibrosis, Am. J. Respir. Cell Mol. Biol. 45 (2011) 72–80, https://doi.org/10.1165/rcmb.2009-0406OC.
[94] M. Dagouassat, N. Suffee, H. Hlawaty, O. Haddad, F. Charni, C. Laguillier, R. Vassy, L. Martin, P.-O. Schischmanoff, L. Gattegno, O. Oudar, A. Sutton, N. Charnaux, Monocyte chemoattractant protein-1 (MCP-1)/CCL2 secreted by hepatic myofibroblasts promotes migration and invasion of human hepatoma cells, Int. J. Canc. 126 (2010) 1095–1108, https://doi.org/10.1002/ijc.24800.
[95] J. Massagué, TGFbeta in cancer, Cell 134 (2008) 215–230, https://doi.org/10.1016/j.cell.2008.07.001.
[96] H. Munir, L.S.C. Ward, H.M. McGettrick, Mesenchymal stem cells as endogenous regulators of inflammation, Adv. Exp. Med. Biol. 1060 (2018) 73–98, https://doi.org/10.1007/978-3-319-78127-3_5.
[97] M.R. Javan, A. Khosrojerdi, S.M. Moazzeni, New insights into implementation of mesenchymal stem cells in cancer therapy: prospects for anti-angiogenesis treatment, Front. Oncol. 9 (2019), https://doi.org/10.3389/fonc.2019.00840.
[98] J. Galipeau, L. Sensébé, Mesenchymal stromal cells: clinical challenges and therapeutic opportunities, Cell Stem Cell 22 (2018) 824–833, https://doi.org/10.1016/j.stem.2018.05.004.
[99] A. Poggi, S. Varesano, M.R. Zocchi, How to hit mesenchymal stromal cells and make the tumor microenvironment immunostimulant rather than immunosuppressive, Front. Immunol. 9 (2018) 262, https://doi.org/10.3389/fimmu.2018.00262.
[100] K.K. Kolluri, G.J. Laurent, S.M. Janes, Mesenchymal stem cells as vectors for lung cancer therapy, Respiration 85 (2013) 443–451, https://doi.org/10.1159/000351284.
[101] X. Wang, H. Chen, X. Zeng, W. Guo, Y. Jin, S. Wang, R. Tian, Y. Han, L. Guo, J. Han, Y. Wu, L. Mei, Efficient lung cancer-targeted drug delivery via a nanoparticle/MSC system, Acta Pharm. Sin. B 9 (2019) 167–176, https://doi.org/10.1016/j.apsb.2018.08.006.
[102] N. de C. Noronha, A. Mizukami, C. Caliári-Oliveira, J.G. Cominal, J.L.M. Rocha, D.T. Covas, K. Swiech, K.C.R. Malmegrim, Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies, Stem Cell Res. Ther. 10 (2019) 131, https://doi.org/10.1186/s13287-019-1224-y.
[103] F.R. Balkwill, M. Capasso, T. Hagemann, The tumor microenvironment at a glance, J. Cell Sci. 125 (2012) 5591–5596, https://doi.org/10.1242/jcs.116392.
[104] H. Hamidi, J. Ivaska, Every step of the way: integrins in cancer progression and metastasis, Nat. Rev. Canc. 18 (2018) 533–548, https://doi.org/10.1038/s41568-018-0038-z.
[105] M.T. Chow, A.D. Luster, Chemokines in cancer, Cancer Immunol Res 2 (2014) 1125–1131, https://doi.org/10.1158/2326-6066.CIR-14-0160.
[106] F. Balkwill, Cancer and the chemokine network, Nat. Rev. Canc. 4 (2004) 540–550, https://doi.org/10.1038/nrc1388.
[107] S.L. Deshmane, S. Kremlev, S. Amini, B.E. Sawaya, Monocyte chemoattractant protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (2009) 313–326, https://doi.org/10.1089/jir.2008.0027.
[108] S.-W. Lai, Y.-S. Liu, D.-Y. Lu, C.-F. Tsai, Melatonin modulates the microenvironment of glioblastoma multiforme by targeting sirtuin 1, Nutrients 11 (2019) 1343, https://doi.org/10.3390/nu11061343.
[109] T. Wang, Q. Zhan, X. Peng, Z. Qiu, T. Zhao, CCL2 influences the sensitivity of lung cancer A549 cells to docetaxel, Oncol Lett 16 (2018) 1267–1274, https://doi.org/10.3892/ol.2018.8769.
[110] L. Li, Y.-D. Liu, Y.-T. Zhan, Y.-H. Zhu, Y. Li, D. Xie, X.-Y. Guan, High levels of CCL2 or CCL4 in the tumor microenvironment predict unfavorable survival in lung adenocarcinoma, Thoracic Cancer 9 (2018) 775–784, https://doi.org/10.1111/1759-7714.12643.
[111] S. Rivas-Fuentes, A. Salgado-Aguayo, S. Pertuz Belloso, P. Gorocica Rosete, N. Alvarado-Vásquez, G. Aquino-Jarquin, Role of chemokines in non-small cell lung cancer: angiogenesis and inflammation, J. Canc. 6 (2015) 938–952, https://doi.org/10.7150/jca.12286.
[112] X. Zhang, X. Qin, C.Y. Qin, Y. Yin, Y. Chen, H. Zhu, Expression of monocyte chemoattractant protein-1 and CC chemokine receptor 2 in non-small cell lung cancer and its significance, Cancer Immunol. Immunother. 62 (2013) 563–570, https://doi.org/10.1007/s00262-012-1361-y.
[113] D. Lambrechts, E. Wauters, B. Boeckx, S. Aibar, D. Nittner, O. Burton, A. Bassez, H. Decaluwé, A. Pircher, K.V. den Eynde, B. Weynand, E. Verbeken, P.D. Leyn, A. Liston, J. Vansteenkiste, P. Carmeliet, S. Aerts, B. Thienpont, Phenotype molding of stromal cells in the lung tumor microenvironment, Nat. Med. 24 (2018) 1277–1289, https://doi.org/10.1038/s41591-018-0096-5.
[114] M. Tsubaki, T. Takeda, Y. Tomonari, K. Mashimo, Y.-I. Koumoto, S. Hoshida, T. Itoh, M. Imano, T. Satou, K. Sakaguchi, S. Nishida, The MIP-1α autocrine loop contributes to decreased sensitivity to anticancer drugs, J. Cell. Physiol. 233 (2018) 4258–4271, https://doi.org/10.1002/jcp.26245.
[115] X. Yu, Y. Pan, H. Ma, W. Li, Simvastatin inhibits proliferation and induces apoptosis in human lung cancer cells, Oncol. Res. 20 (2013) 351–357, https://doi.org/10.3727/096504013X13657689382897.
[116] J. Alizadeh, A.A. Zeki, N. Mirzaei, S. Tewary, A.R. Moghadam, A. Glogowska, P. Nagakannan, E. Eftekharpour, E. Wiechec, J.W. Gordon, F.Y. Xu, J.T. Field, K.Y. Yoneda, N.J. Kenyon, M. Hashemi, G.M. Hatch, S. Hombach-Klonisch, T. Klonisch, S. Ghavami, Mevalonate cascade inhibition by simvastatin induces the intrinsic apoptosis pathway via depletion of isoprenoids in tumor cells, Sci. Rep. 7 (2017) 1–14, https://doi.org/10.1038/srep44841.
[117] F. Wang, W. Liu, J. Ning, J. Wang, Y. Lang, X. Jin, K. Zhu, X. Wang, X. Li, F. Yang, J. Ma, S. Xu, Simvastatin suppresses proliferation and migration in non-small cell lung cancer via pyroptosis, Int. J. Biol. Sci. 14 (2018) 406–417, https://doi.org/10.7150/ijbs.23542.
[118] H.Y. Lee, I.K. Kim, H.I. Lee, J.Y. Mo, C.D. Yeo, H.H. Kang, H.S. Moon, S.H. Lee, The apoptotic effect of simvastatin via the upregulation of BIM in nonsmall cell lung cancer cells, Exp. Lung Res. 42 (2016) 14–23, https://doi.org/10.3109/01902148.2015.1125970.
[119] Y. Li, J. Fu, X. Yuan, C. Hu, Simvastatin inhibits the proliferation of A549 lung cancer cells through oxidative stress and up-regulation of SOD2, Pharmazie 69 (2014) 610–614.
[120] M. Tsubaki, K. Mashimo, T. Takeda, T. Kino, A. Fujita, T. Itoh, M. Imano, K. Sakaguchi, T. Satou, S. Nishida, Statins inhibited the MIP-1α expression via inhibition of Ras/ERK and Ras/Akt pathways in myeloma cells, Biomed. Pharmacother. 78 (2016) 23–29, https://doi.org/10.1016/j.biopha.2015.12.017.
[121] A. Poggi, M.R. Zocchi, Modulating mesenchymal stromal cell function with cholesterol synthesis inhibitors, https://doi.org/10.2174/092986711798184244, (2011).