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Selective JAK/STAT3 signalling regulates transcription of colony stimulating factor-2 and -3 in Concanavalin-A-activated mesenchymal stromal cells

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ABSTRACT

Human bone marrow-derived mesenchymal stromal cells (MSCs) express Toll-like receptors (TLRs) and produce cytokines and chemokines, all of which contribute to these cells' immunomodulatory and pro-angiogenic properties. Among the secreted cytokines, colony-stimulating factors (CSFs) regulate angiogenesis through activation of endothelial cell proliferation and migration. Since MSC are recruited within hypoxic tumors where they signal paracrine-regulated angiogenesis, the aim of this study was to evaluate which CSF members are expressed and are inducible in activated MSC. Furthermore, we investigated the JAK/STAT signal transducing pathway that may impact on CSF transcription. MSC were activated with Concanavalin-A (ConA), a TLR-2/6 agonist as well as a membrane type-1 matrix metalloproteinase (MT1-MMP) inducer, and we found increased transcription of granulocyte macrophage-CSF (GM-CSF, CSF-2), granulocyte CSF (G-CSF, CSF-3), and MT1-MMP. Gene silencing of either STAT3 or MT1-MMP prevented ConA-induced phosphorylation of STAT3, and reversed ConA effects on CSF-2 and CSF-3. Treatment with the Janus Kinase (JAK)2 inhibitor AG490 antagonized the ConA induction of MT1-MMP and CSF-2, while the pan-JAK inhibitor Tofacitinib reversed ConA-induced CSF-2 and -3 gene expression. Silencing of JAK2 prevented the ConA-mediated increase of CSF-2, while silencing of JAK1, JAK3 and TYK2 prevented the increase in CSF-3. Given that combined TLR-activation and locally-produced CSF-2 and CSF-3 could regulate immunomodulation and neovascularization, pharmacological targeting of TLR-2/6-induced MT1-MMP/JAK/STAT3 signalling pathway may prevent MSC contribution to tumor development.

1. Introduction

Mesenchymal stromal cells (MSCs) ability to suppress the immune system is, in part, attributed to Toll-like receptors (TLRs) activation and signalling [1]. In fact, TLR ligation has been reported to influence MSC differentiation, proliferation, migration, and immunomodulation [2,3]. Among the emerging important TLR modulators, naturally-occurring plant lectins are used by the pharmaceutical industry for reliable *in vitro* cell functional assays to study biological systems ranging from mitogenicity to pro-inflammatory cytokine production [4,5]. To this end, the lectin from *Canavalia ensiformis* (Concanavalin-A, ConA) is commonly used to stimulate TLR2/6 [6] signalling, and has helped unravel several MSC angiogenic [7], proliferative [8] and immunosuppressive/immunomodulatory functions [9], as well as their possible use in tissue engineering and cell therapy [10]. More recently, ConA was found to trigger inflammatory responses through JAK/STAT3 signalling in MSC [11]. Thus the first aim of this study was to specifically assess CSF gene expression levels and the contribution of the JAK/STAT signalling pathway that may regulate CSF transcription in ConA-activated MSC.

Recruitment of MSC by experimental implanted vascularizing tumors and their incorporation within the tumor architecture [12,13] implies that these cells must ultimately respond to inflammation- and tumor-derived growth factor cues [14,15]. Given their intrinsic immunosuppressive property [16,17], it is further hypothesized that MSC could contribute to tumor formation and growth *in vivo* through some paracrine-mediated processes involving, in part, promotion of neovascularization [18]. In fact, MSC's contribution to pathological angiogenesis, such as that involved in solid tumor development, has been demonstrated to occur through their integration into tumor vessel walls as pericytes-like MSC [19]. On the other hand MSC were also shown to promote physiological angiogenesis in therapeutic approaches [20,21].

Abbreviations: ConA, Concanavalin-A; CSF, colony stimulating factor; ECM, extracellular matrix; JAK, janus kinase; MSC, mesenchymal stromal cells; MT1-MMP, membrane type-1 matrix metalloproteinase; STAT, signal transducers and activators of transcription; TLRs, Toll-like receptors.

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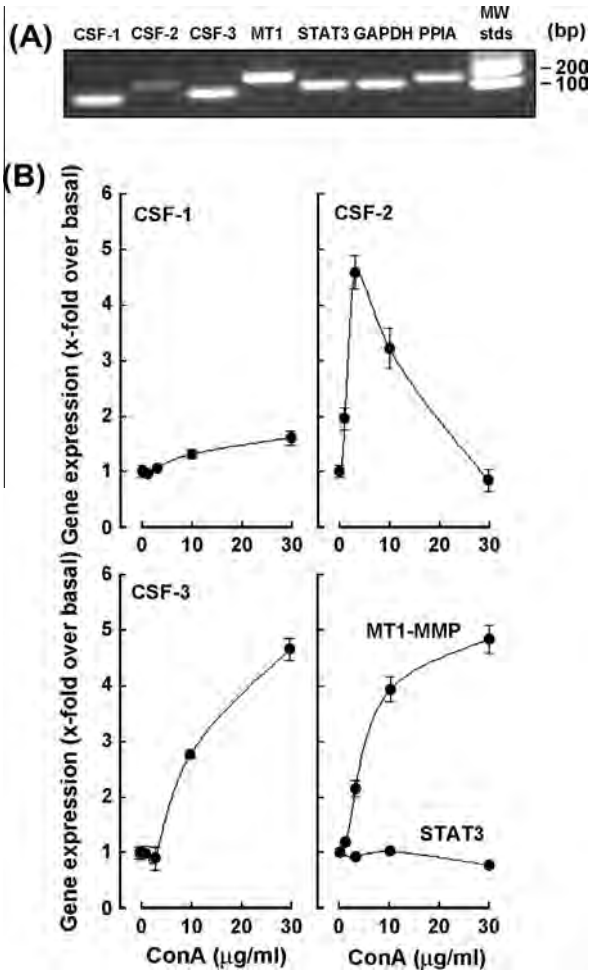

Fig. 1. Concanavalin-A transcriptional regulation of CSF-1, CSF-2, and CSF-3 gene expression. Subconfluent MSC were serum-starved and treated with various concentrations of Concanavalin-A (ConA) for 24 h. Total RNA was extracted, and qRT-PCR was used to assess gene expression. (A) A representative agarose gel of the corresponding single product amplicons is shown and (B) a representative qPCR profile, out of three independent experiments, is shown for the corresponding genes. Data represent mean values from triplicates.

Several mechanisms by which MSC contribute to angiogenesis are currently explained by their significant paracrine effects rather than their differentiation capacity [22]. For example, MSC demonstrate trophic effects via the production of various growth factors and cytokines [23]. These include an assortment of hematopoietic cytokines including constitutively expressed macrophage colony-stimulating factor (M-CSF, CSF-1), stem cell factor, interleukin-6, interleukin-11, inducible expressed granulocyte colony-stimulating factor (G-CSF, CSF-3) and granulocyte macrophage colony-stimulating factor (GM-CSF, CSF-2) [24]. Interestingly, both CSF-2 and CSF-3 induce endothelial cells to trigger an activation/differentiation program related to angiogenesis [25].

ConA treatment further elicites biological lectin/carbohydrate interactions known to mediate extracellular matrix (ECM) protein recognition in part through MT1-MMP, a cell surface matrix metalloproteinase also demonstrated to drive MSC mobilization [7,26], and to trigger crucial signal transducing functions, which included interactions with intracellular p130Cas [27], MTCBP-1 [28], 3BP2 [29], and Src-mediated events [30]. In order to assess the potential triggering events involved in ConA-mediated CSF transcriptional regulation, we also evaluated alternate downstream signalling targets of MT1-MMP such as the Janus Kinase family JAK1, JAK2, JAK3, and TYK2 signalling axis in CSF transcriptional regulation.

2. Materials and methods

2.1. Materials

Sodium dodecylsulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON). Cell culture media were obtained from Life Technologies (Burlington, ON). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, ON). The enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Baie d'Urfé, QC). Micro bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). The JAK family tyrosine kinase inhibitors Tofacitinib (CP-690550) were from Cederlane (Burlington, ON) and AG490 from Calbiochem (La Jolla, CA). The anti-STAT3 (79D7) and anti-phospho-STAT3 (Tyr 705) polyclonal antibodies were from Cell Signalling Technology (Beverly, MA). The polyclonal antibody against the MT1-MMP catalytic domain was from Millipore (Billerica, MA).

2.2. Cell cultures

Human MSC were obtained from marrow biopsies of volunteers undergoing hip replacement and isolated by Ficoll gradient. Cells were plated in high glucose Dulbecco's modified Eagle's medium (DMEM; GibcoBRL) supplemented with 10% inactivated fetal bovine serum (iFBS) (Hyclone Laboratories, Logan, UT) and 100 units/ml Penicillin/Streptomycin. After 5–7 days of incubation in a humidified incubator at 37 °C with 5% CO_2, the nonadherent hematopoietic cells were discarded. Adherent MSC were further grown on non-coated culture dishes and maintained over 14 passages. Analysis by flow cytometry performed at passage 14 revealed that MSC expressed CD44, yet were negative for CD45, CD31, KDR/flk1 (VEGF-R2), flt-4 (VEGF-R3), and Tie2 (angiopoietin receptor) (data not shown). Serum starvation was performed by culturing the cells in high αMEM, 2 mM L-glutamine, and 100 units/ml Penicillin/Streptomycin from which the inactivated fetal bovine serum was omitted.

2.3. Immunoblotting procedures

Cells from MSC were lysed and proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE). After electrophoresis, proteins were electrotransferred to polyvinylidene difluoride membranes and immunoreactive material was visualized by enhanced chemiluminescence as described previously [31].

2.4. Gelatin zymography

Gelatin zymography was used to assess the extracellular levels of secreted proMMP-2 and MMP-2 activities. Briefly, an aliquot (20 μl) of the culture medium was subjected to SDS–PAGE in a gel containing 0.1 mg/ml gelatin (Sigma–Aldrich Canada, G2625). The gels were then incubated in 2.5% Triton X-100 (Bioshop, TRX506.500) and rinsed in nanopure distilled water. Gels were further incubated at 37 °C for 20 h in 20 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35, 50 mM Tris–HCl buffer, pH 7.6 and then stained with 0.1% Coomassie Brilliant blue R-250 (Bioshop, CBB250) and destained in 10% acetic acid, 30% methanol in water. Gelatinolytic activity was detected as unstained bands on a blue background.

2.5. Total RNA isolation, cDNA synthesis and real-time quantitative RT-PCR

Total RNA was extracted from MSC monolayers using TRIzol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 1 μg of total RNA was reverse-transcribed into cDNA using a high

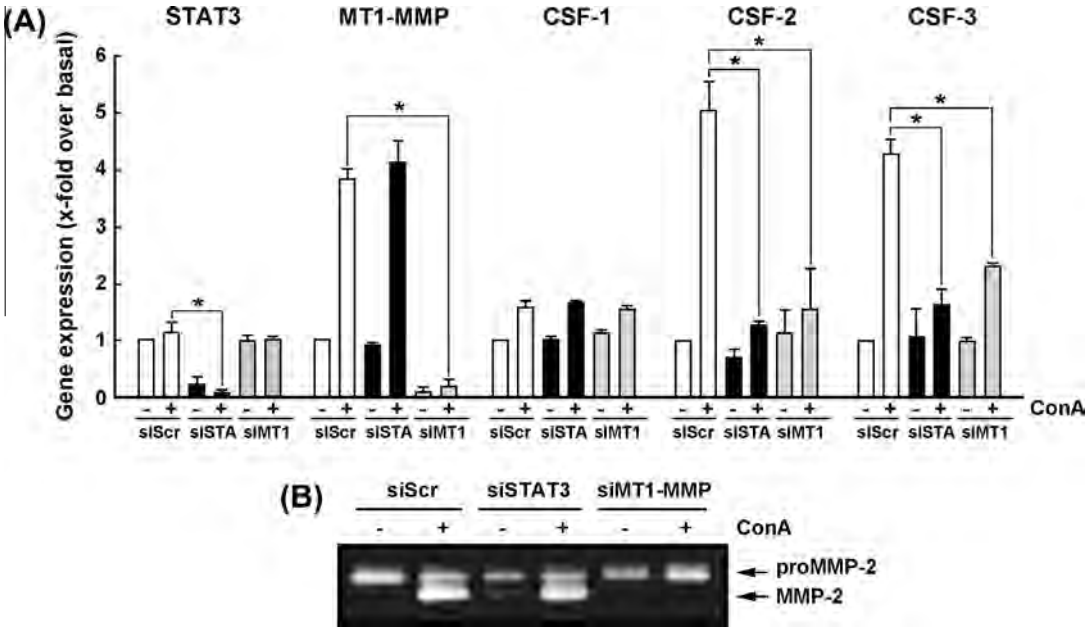

Fig. 2. MT1-MMP or STAT3 gene silencing abrogates ConA-induced CSF-2, and CSF-3 transcriptional regulation. MSC were transiently transfected with scrambled sequences (siScr), STAT3 siRNA (siSTA), or MT1-MMP siRNA (siMT1) as described in the Methods section. (A) Total RNA was extracted, and qRT-PCR was used to assess STAT3, MT1-MMP, CSF-1, CSF-2, and CSF-3 gene expression upon treatment with or without 30 μg/ml of Concanavalin-A (ConA) for 24 h and (B) conditioned media was isolated from the serum-starved and ConA-treated transfected cells, and gelatin zymography performed as described in the Methods section to assess the extent of proMMP-2 activation.

capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80 °C prior to PCR. Gene expression was quantified by real-time quantitative PCR using iQ SYBR Green Supermix (BIO-RAD, Hercules, CA). DNA amplification was carried out using an Icycler iQ5 (BIO-RAD, Hercules, CA) and product detection was performed by measuring binding of the fluorescent dye SYBR Green I to double-stranded DNA. The following primer sets were provided by QIAGEN (Valencia, CA): MT1-MMP (Hs_MMP14_1_SG, QT00001533), β-Actin (Hs_ACTB_2_SG, QT01680476), GAPDH (Hs_GAPDH_1_SG, QT00079247), PPIA (Hs_PPIA_4_SG, QT01866137), STAT3 (Hs_STAT3_1_SG, QT00068754), CSF-1 (Hs_CSF1_1_SG, qt00035224), CSF-2 (Hs_CSF2_1_SG, qt00000896), CSF-3 (Hs_CSF3_1_SG, qt00001414). The relative quantities of target gene mRNA against an internal control, β-Actin/GAPDH/PPIA RNA, were measured by following a ΔC_T method employing an amplification plot (fluorescence signal *vs*. cycle number). The difference (ΔC_T) between the mean values in the triplicate samples of target gene and those of β-Actin/GAPDH/PPIA RNA were calculated by iQ5 Optical System Software version 2.0 (BIO-RAD, Hercules, CA) and the relative quantified value (RQV) was expressed as $2_T^{-\Delta C}$. Semi-quantitative PCR was performed to examine amplification products and amplicons resolved on 1.8% agarose gels containing 1 μg/ml ethidium bromide.

2.6. Transfection method and RNA interference

Subconfluent (30–50%) MSC were transiently transfected for 24 h in serum-free media and with 20 nM siRNA against STAT3 (human Hs_STAT3_7 FlexiTube siRNA, SI02662338), MT1-MMP (human Hs_MMP14_6 HP siRNA, SI03648841), JAK1 (human Hs_JAK1_5 FlexiTube siRNA, SI00605514), JAK2 (human Hs_JAK2_7 FlexiTube siRNA, SI02659657), JAK3 (human Hs_JAK3_5 FlexiTube siRNA, SI00604800), TYK2 (HS_TYK2_5 FlexiTube siRNA, SI02223221), or scrambled sequences (AllStar Negative Control siRNA, 1027281) using Lipofectamine 2000 transfection reagent (Invitrogen, CA). Small interfering RNA and mismatch siRNA were synthesized by QIAGEN and annealed to form duplexes.

2.7. Statistical data analysis

Data are representative of three or more independent experiments. Statistical significance was assessed using Student's unpaired *t*-test. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance in the figures.

3. Results

3.1. Concanavalin-A transcriptional regulation of CSF-1, CSF-2, and CSF-3 gene expression

We first assessed whether each of the CSF-1, CSF-2, and CSF-3 transcripts were present in MSC. Total RNA was extracted from untreated MSC and cDNA was reversed transcribed as described in the Methods section. qPCR was then performed and end of cycle products were loaded onto an agarose gel to demonstrate that a single amplicon with the expected size was produced (Fig. 1A). When serum-starved MSC were treated with various ConA concentrations for 18 h, we found that CSF-1 gene expression was only moderately induced, while that of CSF-2 and CSF-3 were significantly upregulated, with CSF-2 reaching a peak at 3 μM ConA and CSF-3 reaching maximal stimulation at 30 μM ConA (Fig. 1B). Given that ConA is a well-documented inducer of MT1-MMP, we also observed a dose-dependent increase in MT1-MMP gene expression as expected, while that of STAT3 remained unaffected (Fig. 1B).

3.2. MT1-MMP and STAT3 gene silencing abrogates ConA-induced CSF-2 and CSF-3 transcriptional regulation

As MT1-MMP is well known to be induced by ConA and to transduce intracellular-mediated signalling in processes such as invasion [32], inflammation [33] and autophagy [34], and since STAT3 is believed to partly regulate CSF transcription [35], we used gene silencing strategies to evaluate their effects in ConA-treated

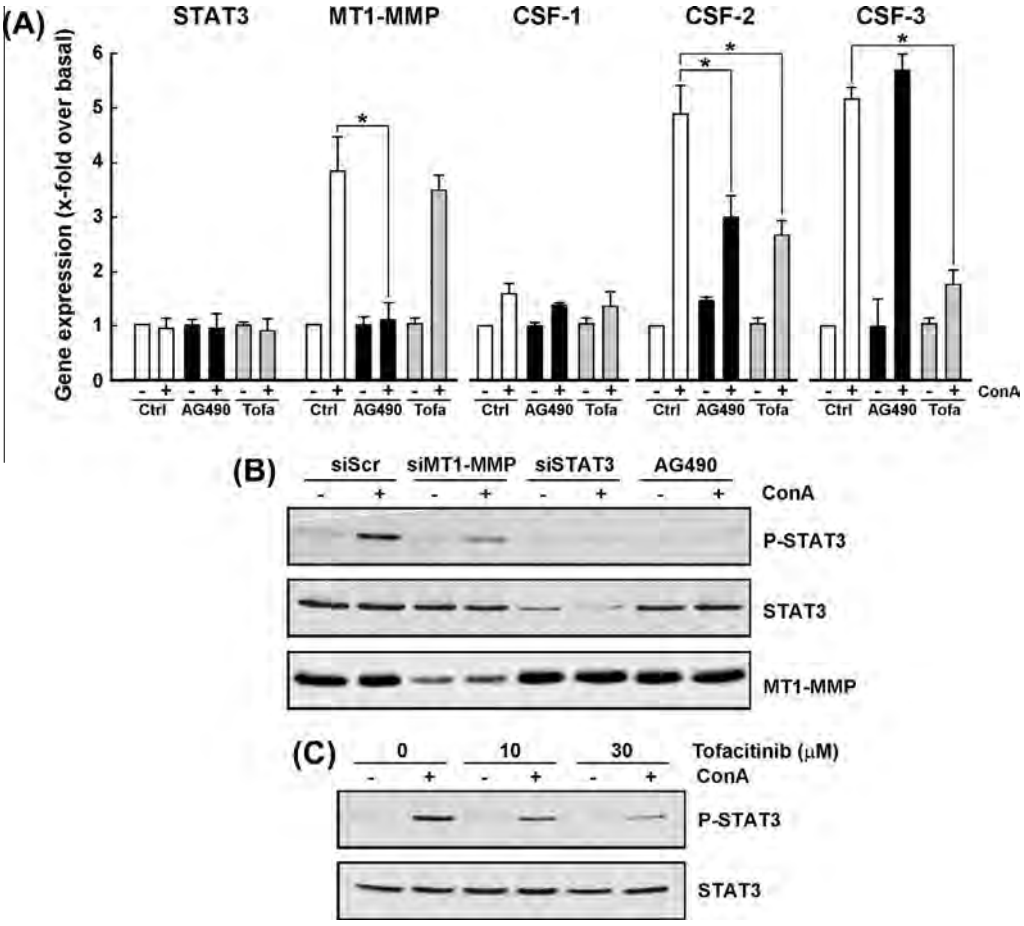

Fig. 3. Pharmacological inhibition of JAK family members selectively antagonizes ConA-mediated increases of MT1-MMP, CSF-1, -2, and -3. (A) Subconfluent MSC were serum-starved and treated with 10 μM of the JAK2 inhibitor AG490 (black bars) or the pan-JAK inhibitor Tofacitinib (grey bars), in the presence or absence of 30 μg/ml Concanavalin-A (ConA) for 24 h. Total RNA was extracted, and qRT-PCR was used to assess gene expression, (B) MT1-MMP and STAT3 gene silencing was performed as described in the Methods section, and then cells were treated with 30 μg/ml ConA for 1 h. Cell lysates were isolated, then Western blotting and immunodetection were performed with anti-STAT3, anti-phosphoSTAT3, and anti-MT1-MMP antibodies as described in the Methods section, and (C) cells were pre-incubated for 30 min in the presence of Tofacitinib and then treated with ConA for 1 h. Cell lysates were isolated, and then Western blotting and immunodetection were performed with anti-STAT3 and anti-phosphoSTAT3 antibodies as described in the Methods section.

cells. MSC were transiently transfected with siRNA against STAT3 (siSTA),or MT1-MMP (siMT1), or with a scrambled sequence (siScr), and then treated with ConA. STAT3 and MT1-MMP gene silencing were very specific and effective (Fig. 2A, STAT3 black bars, MT1-MMP grey bars). We found that diminishing either STAT3 or MT1-MMP expression prevented ConA from inducing CSF-2 and CSF-3 gene expression without affecting the marginal increase in CSF-1 (Fig. 2A). STAT3 gene silencing did not reverse MT1-MMP-dependent proMMP-2 activation, as shown by the zymogram of the conditioned media (Fig. 2B). Collectively, this suggests that potential STAT3 transcription factor binding sites may be crucial within the CSF promoter regions and may regulate their transcription.

3.3. Pharmacological inhibition of JAK family members selectively antagonizes ConA-mediated increases of MT1-MMP, CSF-1, -2, and -3

We recently reported a JAK/STAT3 signalling axis in ConA-treated MSC [11]. We therefore assessed whether treatment with the pan-JAK inhibitor Tofacitinib or with the JAK2 inhibitor AG490 could affect ConA-mediated CSF transcriptional regulation. We found that neither ConA-induced CSF-1 nor ConA-induced CSF-3 gene expression were affected by AG490 (Fig. 3A, black bars); only ConA-induced CSF-2 and ConA-induced MT1-MMP gene expression were inhibited. When Tofacitinib was used, diminished ConA induction of CSF-2 and CSF-3 were observed (Fig. 3A, grey bars). These results suggest that a potential JAK2/STAT signalling axis regulates ConA-mediated transcription of CSF-2 and MT1-MMP, and that alternate JAK signalling is required for CSF-2 and CSF-3 gene expression. In order to validate functional inhibition by AG490 and Tofacitinib, monitoring of the STAT3 phosphorylation state was then performed by immunoblotting. Optimal STAT3 phosphorylation has already been reported to occur after 1–2 h treatment [11]. We observed that treatment with the JAK2 inhibitor AG490 or gene silencing of either MT1-MMP or STAT3 abrogated ConA-induced STAT3 phosphorylation (Fig. 3B). Treatment with the pan-JAK inhibitor Tofacitinib also antagonized ConA-induced STAT3 phosphorylation (Fig. 3C). Collectively, these observations lead us to conclude that selective JAK family members are required in the transcriptional regulation of CSF.

3.4. Evidence for selective JAK involvement in ConA-induced CSF-2 and CSF-3 gene expression

Given the above evidence of some Janus Kinase family members' implication in the transcriptional regulation of CSF, we next specifically silenced each of the JAK1, JAK2, JAK3, and TYK2 members using siRNA strategy with silencing efficiency ranging from

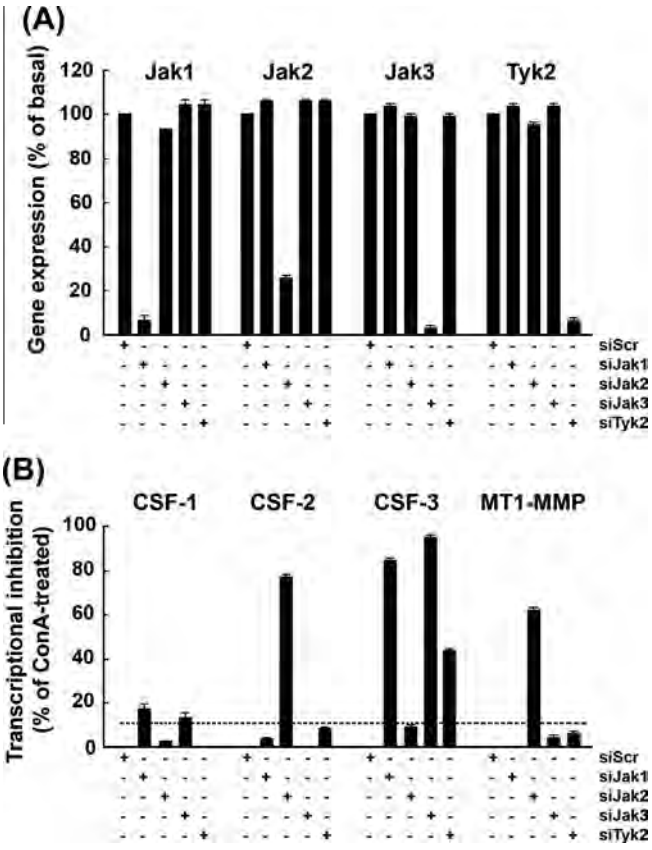

Fig. 4. Evidence for selective JAK involvement in ConA-induced CSF-2 and CSF-3 gene expression. JAK1, JAK2, JAK3, and TYK2 gene silencing was performed as described in the Methods section. Total RNA was extracted, and qRT-PCR was used to assess (A) silencing efficacy and (B) impact upon ConA-induced CSF-1, CSF-2, CSF-3, and MT1-MMP gene expression.

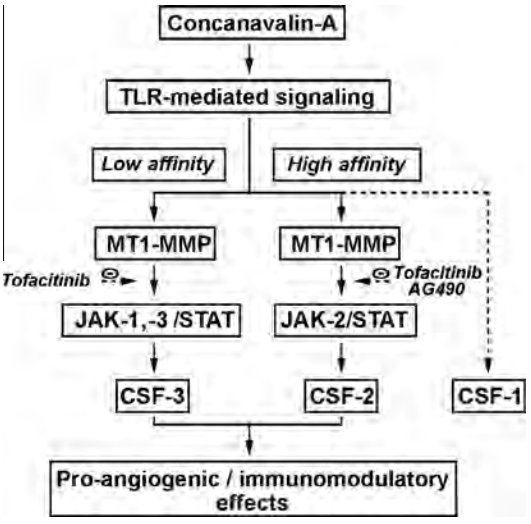

Fig. 5. Schematic representation of the signalling partners involved in the transcriptional regulation of angiogenic CSF-2 and -3. Activation of MSC with Concanavalin-A (ConA) triggers cell surface-mediated Toll-like receptor (TLR) signalling. High ConA concentrations (low affinity effect) promotes MT1-MMP-mediated CSF-3 transcriptional induction through a JAK-1,-3/STAT pathway. On the other hand, low ConA concentrations (high affinity effect) promote MT1-MMP-mediated CSF-2 transcriptional regulation through a JAK-2/STAT pathway. ConA-mediated CSF-2 and -3 is thought to contribute to MSC's pro-angiogenic and immunomodulatory effects. High ConA concentrations also contribute, to some extent, to CSF-1 transcriptional regulation through a MT1-MMP/JAK/STAT-independent pathway.

78% to 96% (Fig. 4A). SiRNA-transfected and serum-starved cells were next treated with ConA and qRT-PCR was performed to monitor the impact of silencing each JAK member on the induction of CSF-1, CSF-2, CSF-3, and MT1-MMP gene expression. Regardless of which JAK member was silenced, we found very low (5–18%) diminution of ConA-induced CSF-1 gene expression (Fig. 4B). In contrast, silencing of JAK2 significantly abrogated ConA-induced CSF-2 and MT1-MMP, while silencing of JAK1 and of JAK3 significantly decreased ConA-induced CSF-3 gene expression. TYK2 silencing only moderately impacted ConA-induced CSF-3 (Fig. 4B).

4. Discussion

Angiogenic activity of classical hematopoietic cytokines has been reported in different experimental models, including wound healing, chronic inflammation, and tumor growth [36]. Interestingly, recent identification and functional study of cytokines and chemokines involved in tumorigenesis confirm that MSC exert paracrine regulation of angiogenesis, tumor cell proliferation and apoptosis [37], and may affect cancer progression through various cell signalling pathways [38]. In parallel, accumulating evidence supports the involvement of MSC in cancer pathogenesis, as reflected by their homing to vascularizing tumors and adaptive capacity to a hypoxic environment [14,35]. Although the impact of these contributions remains poorly understood, MSC are becoming further appreciated as critical components of the tumor microenvironment where they also suppress antitumor immune responses [39,40]. Given the immunomodulatory and proangiogenic functions exerted by cytokines and the roles that MSC play in brain tumor development, we provide evidence that a subset of cytokines from the CSF family may significantly contribute to the paracrine angiogenic/immunomodulatory properties of MSC.

In the current study, we also specifically provide evidence for transcriptional regulation of CSF-2 and CSF-3 in activated MSC paracrine proangiogenic phenotype. Interestingly, the vasculogenic and angiogenic properties of MSC have already been inferred [41], but the exact paracrine mechanisms unknown. Here, we confirm that CSF-2 and CSF-3 gene expression is increased upon MSC activation by ConA. Given CSF-2 and CSF-3 are known inducers of endothelial cells [25], one may confidently infer that the increases observed in MSC in CSF may, in part, explain their paracrine proangiogenic phenotype. This is supported by the fact that CSF-2 acts as an inducer of endothelial cell migration and proliferation related to angiogenesis [25], and that it induces angiogenesis in rat connective tissue by a direct effect on endothelial cells or by the recruitment and activation of macrophages that release their own angiogenic factors [42]. In support of this, subnanomolar concentrations of CSF-2 and CSF-3 were found to induce the proliferation of endothelial cells derived from human vessels [43], and from murine microvascular capillaries [44]. Suggestion of an autocrine growth regulation by CSF-2 and CSF-3 was also reported in human gliomas [45]. In fact, CSF-2 has recently been confirmed to have an effective role in the evolution and pathogenesis of high grade gliomas which also express high levels of the CSF-2 receptor [46], whereas sustained proliferative signalling from CSF-3 was recently inferred to contribute to glioma genesis and recurrence [47]. In the present study, we demonstrate that lectin-activated MSC promotes CSF transcription through selective JAK/STAT3 signalling.

STAT transcription factors are mostly dedicated to hematopoiesis and immunity, and crosstalk between JAK/STAT and TLR has been inferred [48] in several MSC functions intrinsically linked to TLR signalling and immune crosstalk [1]. Concanavalin-A is documented as a TLR-2 and TLR-6 agonist. Accordingly, TLR-2 and TLR-6 gene expression was confirmed in previous reports [6]. How their expression specifically affects ConA-induced CSF-2 and

CSF-3 transcription will need further experimentation. Preliminary results from our laboratory suggest that gene silencing of either TLR-2 or TLR-6 abrogated ConA-mediated induction of CSF-2 and of CSF-3 (not shown). Given that ConA-activated MSC were previously shown to involve MT1-MMP intracellular signalling, we now further define an unreported intracellular signalling function of MT1-MMP in the regulation of cytokine expression requiring STAT3 as an intermediate. This links both MT1-MMP's intracellular domain-mediated signalling function to its intrinsic extracellular domain-mediated matrix hydrolysis and promotion of cell migration. This may also, in part, explain some of the immunosuppressive properties of activated MSC, which ultimately concurs to facilitate MSC recruitment by a tumor and evasion from the immune system.

Membrane-bound MMPs, such as MT1-MMP, have recently become critical pharmacological targets in angiogenesis-related disease treatment [49]. In light of our data, one can therefore envision that targeting the MT1-MMP/JAK/STAT signaling axis may also significantly impact on MSC paracrine angiogenic properties. Accordingly, preclinical proof of principle rationale was recently provided for the design and development of novel and selective MT1-MMP inhibitors [50,51], while impact on lymphangiogenesis with selective targeting of MT1-MMP was also recently achieved through the use of specific monoclonal antibodies [52]. How these inhibitors of MT1-MMP catalytic and signalling functions will remain to be better investigated with relation to any transcriptional regulation.

Interestingly, ConA-mediated activation of MSC was shown to trigger cyclooxygenase (COX)-2 expression [11], which ultimate end-product metabolite prostaglandin E2 is involved in the inhibition of cytokine release by T cells [53] and of T cell activation and proliferation [54]. MT1-MMP involvement in the major histocompatibility complex (MHC) class I was also recently suggested. Interestingly, phenotypic characterization of MSC by flow cytometry showed expression of MHC class I alloantigens [55]. Shedding of the tumor cell surface MHC class I chain-related molecule A by MT1-MMP was demonstrated to regulate sensitivity of tumor cells to NK cell killing, a process which may add to tumor immune evasion by MSC and contribute to tumor progression [56]. Such cell surface proteolytic activity of MT1-MMP was also shown in MSC to contribute to cleavage of CD44, another cell adhesion molecule expressed at the cell surface of MSC, and known to promote cell migration [57,58].

In conclusion, along with the demonstrated existence of a signalling axis linking MT1-MMP to the JAK/STAT pathway in MSC [11], this study further unravels the impact that this signalling axis may exert on CSF transcriptional regulation on the paracrine pro-angiogenic and immunomodulatory properties of MSC. We further identified the JAK family members involved in selective transcriptional regulation of CSF-2 and CSF-3 (Fig. 5). Our data now allow the potential design and assessment of multi-targeted inhibitory approaches of the JAK/STAT pathway which may thereof offer greater therapeutic efficacy to counteract MSC angiogenic/immunomodulatory contributions to brain tumor development.

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