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Impact of Mesenchymal Stem Cell secreted PAI-1 on colon cancer cell migration and proliferation

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ABSTRACT

Mesenchymal Stem Cells are known to engraft and integrate into the architecture of colorectal tumours, with little known regarding their fate following engraftment. This study aimed to investigate mediators of Mesenchymal Stem Cell (MSC) and colon cancer cell (CCC) interactions. Mesenchymal Stem Cells and colon cancer cells (HT29 and HCT-116) were cultured individually or in co-culture on 3-dimensional scaffolds. Conditioned media containing all secreted factors was harvested at day 1, 3 and 7. Chemokine secretion and expression were analyzed by Chemi-array, ELISA (Macrophage migration inhibitory factor (MIF), plasminogen activator inhibitor type 1 (PAI-1)) and RQ-PCR. Colon cancer cell migration and proliferation in response to recombinant PAI-1, MSCs and MSCs + antibody to PAI-1 was analyzed using Transwell inserts and an MTS proliferation assay respectively.

Chemi-array revealed secretion of a wide range of factors by each cell population, including PAI-1and MIF. ELISA analysis revealed Mesenchymal Stem Cells to secrete the highest levels of PAI-1 (MSC mean 10.6 ng/mL, CCC mean 1.01 ng/mL), while colon cancer cells were the principal source of MIF. MSC-secreted PAI-1 stimulated significant migration of both CCC lines, with an antibody to the chemokine shown to block this effect (67–88% blocking,). A cell-line dependant effect on CCC proliferation was shown for Mesenchymal Stem Cell-secreted PAI-1 with HCT-116 cells showing decreased proliferation at all concentrations, and HT29 cells showing increased proliferation in the presence of higher PAI-1 levels.

This is the first study to identify PAI-1 as an important mediator of Mesenchymal Stem Cell/colon cancer cell interactions and highlights the significant functional impact of Mesenchymal Stem Cell-secreted PAI-1 on colon cancer cells.

1. Introduction

In colon cancer, the induction of a migratory phenotype in cancer cells leads to the development of distant metastases, for which there are limited therapeutic options [1]. Our ability to delineate which tumour characteristics promote this devastating metastatic potential remains limited, and a resultant inability to individualise therapy leads to significant mortality. This highlights an urgent need to expand our relatively limited knowledge of primary tumour biology. Initially, investigation of carcinogenesis focused on epithelial cells alone. However, the role of the stroma has been afforded increasing prominence in recent years and it is now well established that colonic tumours exist in a microenvironment rich in inflammatory cells, immune cells, and stromal cells [2,3]. Mesker at al [4] reported that the epithelial–stromal ratio of colon carcinomas is a predictor of survival independent of lymph node status and tumour stage [4]. As a result, unravelling pathways of stromal–epithelial interactions in colon cancer is crucial to advancing our ability to predict and ultimately prevent metastasis.

One remarkable subset of stromal cells is Mesenchymal Stem Cells (MSCs). MSCs are non-hematopoietic, multipotent cells which are known to be actively recruited to the site of tumours, including colorectal tumours and their metastases [5–7]. Despite this, our knowledge of colon cancer cell-MSC interactions remains limited, and the role of MSCs within colon tumours has yet to be elucidated. Only one recently published study exists in the literature regarding factors mediating CRC/MSC interactions which revealed that paracrine neuregulin-1/HER3 signals initiated by MSCs promote CRC tumour progression [8]. MSCs are known to secrete a wide range of pro-inflammatory chemokines [9] and are therefore

Abbreviations: MSC, Mesenchymal Stem Cell; CCC, colon cancer cell; CRC, colorectal cancer; PAI-1, plasminogen activator inhibitor type 1; MIF, macrophage migration inhibitory factor.

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likely to exert a significant effect on surrounding cells. Indeed, in many disease models, stromal and epithelial cells are thought to interact in a dynamic and bidirectional manner, potentially mediated through secreted cytokines [10]. Two cytokines of particular interest in this study are Macrophage migration inhibitory factor (MIF) and Plasminogen activator inhibitor type 1, both of which have been implicated in colorectal cancer disease progression.

Macrophage migration inhibitory factor (MIF) was among the first identified cytokines and in the past 50 years its roles in immunity, inflammation and tumour growth have been well described [11–13]. In colon cancer, MIF has been shown to promote tumorigenesis and one study demonstrated 20–40 times as many MIF-positive cells in the mucosa of patients with colon cancer than in normal tissue [14]. PAI-1 is a serine protease inhibitor and a component of the plasminogen activator system [15]. Like MIF, PAI-1 levels have been shown to be increased in colorectal cancer tissue when compared with normal tissue [16]. PAI-1, previously shown to regulate cell migration and proliferation of endothelial cells [17], is of particular clinical relevance in colorectal cancer since high serum and tissue levels have been shown to correlate with poor prognosis [16]. Since MSCs are known to be present in colonic tumours and are known to produce pro-inflammatory cytokines, including PAI-1 and MIF [18], this study aimed to investigate cytokine secretion and functional effects of MSCs on colon cancer cells.

2. Methods

2.1. Cell populations

Human colon cancer cell lines (HT29 and HCT-116) were obtained from the American Type Culture Collection and cultured in McCoy's 5A medium, supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml Penicillin/100 μg/ml Streptomycin (Pen/Strep). Human MSCs were obtained through the Regenerative Medicine Institute at NUI Galway. With ethical approval and informed consent, bone marrow was aspirated from the iliac crests of healthy volunteers in accordance with a defined clinical protocol [19,20]. MSCs were isolated from the bone marrow aspirates via direct plating and were then cultured for 12–15 days to deplete the non-adherent haematopoietic cell fraction. The ability of MSCs to differentiate into chondrocytes, adipocytes and osteoblasts was confirmed prior to use. Furthermore, characterisation of surface receptors was performed targeting the markers CD105, CD73, CD90 (positive) and haematopoietic markers CD34, CD45 (negative). MSCs were cultured in α-Minimum Essential Medium (αMEM) supplemented with serotyped 10% FBS and Pen/Strep and were used in assays at passages 4–6.

2.2. Three dimensional cell culture

MSCs, HT29 or HCT-116 were cultured individually or in direct co-culture on three dimensional scaffolds (3D Biotek LLC, New Jersey). Each 3D scaffold was seeded with the same total number of cells, consisting of either colon cancer cells (CCC) or MSCs alone (5.5×10^5 cells) or a 3:1 mix of CCC and MSC (4.13×10^5:1.38×10^5, CCC:MSC). All experiments were carried out in triplicate. For seeding, cell populations alone or in combination were resuspended in a uniform amount of MSC medium (120 μl) and loaded onto the centre of a scaffold in a 12-well plate. Immediately after seeding, the cell droplet occupied approximately 70% of the total scaffold area. The plate was then incubated at 37 °C, 5% CO_2 for 3 h to allow the cell droplet to penetrate the entire scaffold. A uniform amount of MSC medium (1380 μl) was then added to each well. Cells were cultured on scaffolds for up to 7 days with media changed on day 1, 3, 5 and 7.

2.3. Collection of conditioned medium

Cell-conditioned medium (CM) containing all secreted factors from wells containing 3D scaffolds was harvested at day 1, 3 and 7. Media was also replenished on day 5 to ensure uniform time periods for accumulation of secreted products. Cell-conditioned medium was centrifuged at 13,000 rpm for 2 min to remove cellular debris, and stored at −20 °C until required for chemokine analysis.

2.4. Chemokine detection

Chemi-array human cytokine antibody arrays (R&D systems) were used to assess cytokine secretion profiles of CCC and MSCs. This array permitted simultaneous detection of secreted factors in each sample. Cell CM was collected as described and applied to the membranes according to manufacturers'' instructions. Representative medium from each cell line, not exposed to the cells themselves, was also analyzed as a negative control for each experiment. Chemiluminescent images were acquired using FlourChem™ imaging system (Alpha Innotech) and analyzed with Alpha Ease software.

2.5. Elisa

Specific levels of PAI-1 or MIF secreted by each cell population were quantified using Quantikine Enzyme Linked Immunosorbent Assay (ELISA) kits (R&D Systems) according to manufacturers' instructions. The optical density of each well was then read on a microplate reader at 450 nm wavelength (Multiskan RC, Thermo Scientific), and PAI-1 or MIF concentration in each sample determined using the standard curve. Experiments were carried out in triplicate. Each sample was analyzed in duplicate.

2.6. In vitro cell migration assay

Transwell® Permeable Supports (Corning Inc, Sigma–Aldrich) with 8.0 μm pores were used to track migration of CCC (HCT-116, HT29) in response to recombinant standards of PAI-1 or factors secreted by MSCs cultured in the well below (1.0×10^5 cells per well, 24 well plate format), in the presence or absence of an antibody to PAI-1 (10 μg/mL, For 3 h, R&D systems). Basal medium was employed as a negative control and 10% FBS served as a positive control. Migrated cells were stained using haematoxylin and counted in five fields of view per membrane using an Olympus B×60 microscope and image analysis software. Due to the high number of migrated cells, counting was performed at 40× magnification. Each experiment was repeated in triplicate, with results expressed against negative control as Mean ± SEM.

2.7. In vitro proliferation assay

Cell proliferation was assessed using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (MTS, Promega). Colorectal cancer cell lines were seeded into 96-well plates (6×10^4 cells per well) and allowed to adhere overnight. Test substrates included complete media (positive control) and PAI-1 protein standards (5, 10, 15 and 20 ng/mL, R&D systems, For 1 h). Proliferation in response to MSCs ± polyclonal antibody (10 μg/mL, For 3 h, R&D systems) was performed by indirect co-culture with each population separated by a 0.4 μm porous membrane. The MTS assay was then performed and absorbance read in a plate reader at 490 nM (Multiskan RC, Thermo Scientific). Data represent the Mean reading of 8 wells in each experiment (performed in triplicate) and are expressed as Mean ± SEM.

2.8. Real time quantitative PCR

Cells were homogenised in 1 mL of QIAzol Lysis reagent and total RNA isolated using the RNeasy® Mini Kit (QIAGEN), reverse transcribed and amplified by real-time quantitative PCR (RQ-PCR) using ABI Prism 7000 (Applied Biosystems).Taqman® Universal Master Mix and Gene Expression Assays (Applied Biosystems) designed for the target gene PAI-1, and endogenous control genes *Mitochondrial Ribosomal Protein L19* (*MRPL19*) and *Peptidylprolyl Isomerase A* (*PPIA*) were used. Data was expressed in a linear form using the formula $2^{-\Delta\Delta CT}$[21].

3. Results

3.1. Chemokine secretion

Chemi-array analysis of samples at day 7 allowed simultaneous detection of the range of factors secreted by CCCs (Fig. 1A). As previously reported in a number of studies, MSCs in this study were shown to secrete a number of factors including Interleukin-6, MIF and PAI-1 (results not shown) [18]. HT29 and HCT-116 (Fig. 1A) cell lines secreted a similar panel of cytokines including MIF and PAI-1, with HT29 also secreting Interleukin-8. All cell populations studied secreted PAI-1 and MIF. Considering this and their known clinical relevance in colon cancer, MIF and PAI-1 were then selected for ELISA analysis (Fig. 1B). Conditioned media harvested from day 1, 3 and 7 of HT29 cells cultured in 3D contained the highest levels of secreted MIF (mean: 9 ng/mL, range: 4–12.1 ng/mL) with MSCs and HCT-116 secreting lower levels (MSC: mean: 4.36 ng/mL, range: 2–4.7 ng/mL, HCT-116: mean: 3.61 ng/mL, range: 1.5–7.49 ng/mL) (Fig. 1B).

Conversely, MSCs were found to secrete highest levels of PAI-1 (mean: 10.6 ng/mL, range: 3.68–14.65 ng/mL). Although HCT-116 secreted higher levels of PAI-1 than HT29, both CCC lines secreted significantly lower levels of PAI-1 than MSCs (HT29: mean: 0.31 ng/mL, range: 0.03–0.71 ng/mL, HCT-116: mean: 1.72, range: 0.35–2.6 ng/mL) (Fig. 1B).

3.2. Gene expression

The baseline level of PAI-1 gene expression in each cell population was then determined to assess whether it was reflective of protein secretion. PAI-1 gene expression was expressed relative to mean expression of endogenous control genes, with values expressed relative to the lowest expresser (ΔΔCT). HT29 cells expressed the lowest levels of PAI-1 with HCT-116 only 1.7-fold higher (Log_{10} Relative Quantity (RQ): 0.24). Similar to protein secretion, expression of PAI-1 was highest in MSCs compared with CCCs (77-fold higher, log_{10} RQ: 1.89) (results not shown).

3.3. Effect of co-culture on chemokine secretion

Upon co-culture of CCCs with MSCs, no significant change in MIF secretion was observed on day 1 or 3 of culture with HT29 (Fig. 2A) or HCT-116 (Fig. 2B) cells. At day 7, there was an increase in MIF secretion by HT29 cells and MSCs co-cultured at a 3:1 ratio (Fig. 2A). The same pattern was observed upon co-culture of HCT-116 with MSCs although absolute secretion levels were significantly less in this population (Fig. 2B).

Although, as previously outlined, each 3D scaffold contained the same total number of cells (5.5×10^5), upon 3-dimensional co-culture of HT29 (Fig. 3A) or HCT-116 cells (Fig. 3B) with MSCs,

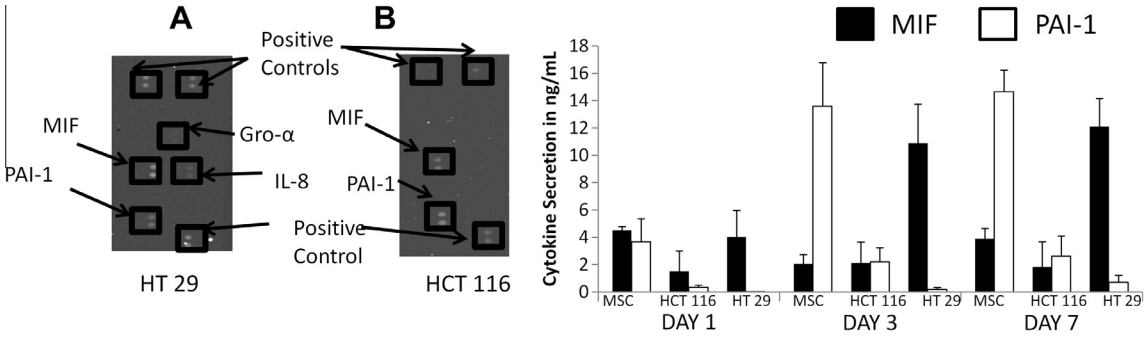

Fig. 1. Analysis of chemokine secretion. (A) Chemi-array analysis of range of factors secreted by HT29 and HCT-116 colon cancer cell lines. (B) Level of PAI-1 and MIF secreted by MSC, HCT-116 and HT29 on day 1, 3 and 7 of individual culture.

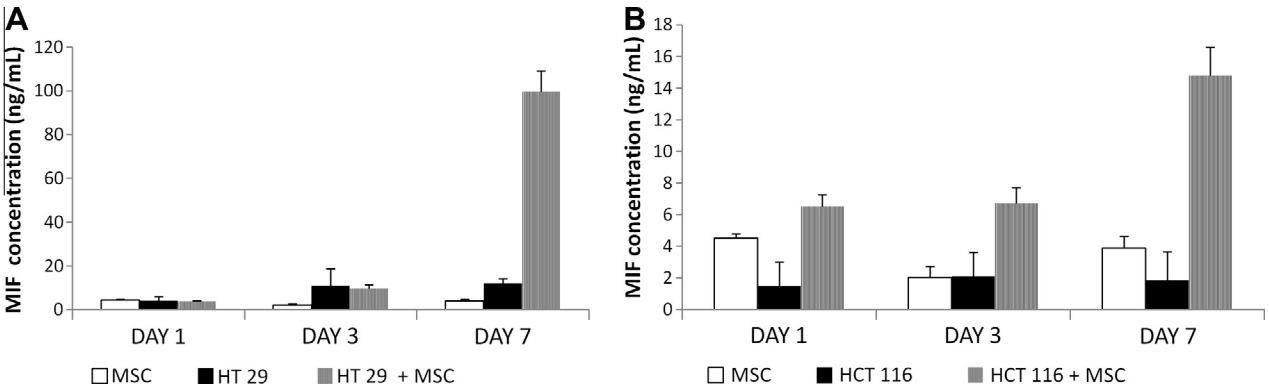

Fig. 2. Effect of co-culture on MIF secretion. MIF secretion upon 3D co-culture of (A) MSC + HT29 and (B) MSC + HCT-116

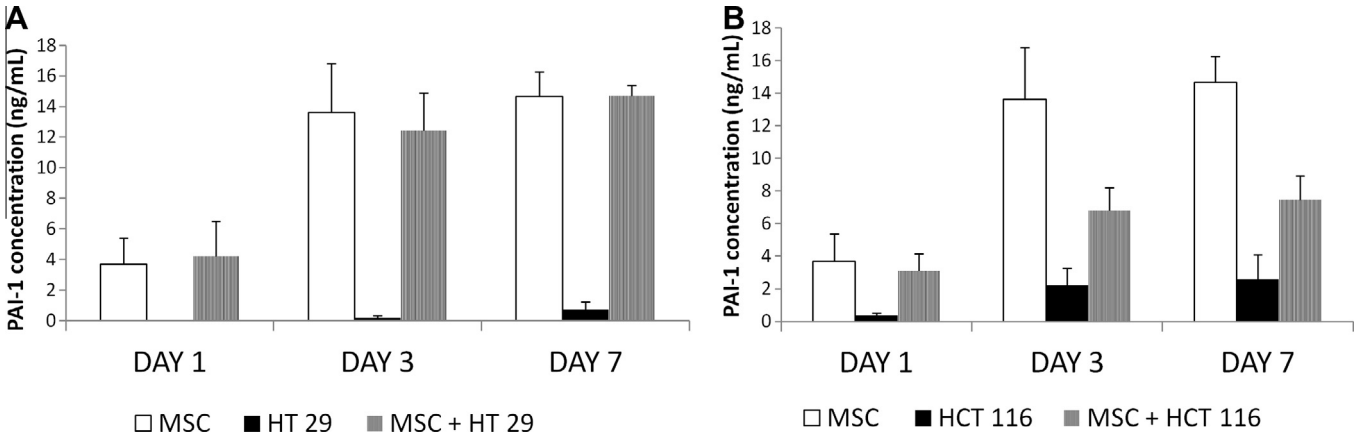

Fig. 3. Effect of co-culture on PAI-1 secretion. PAI-1 secretion upon 3D co-culture of (A) MSC + HT29 and (B) MSC + HCT-116.

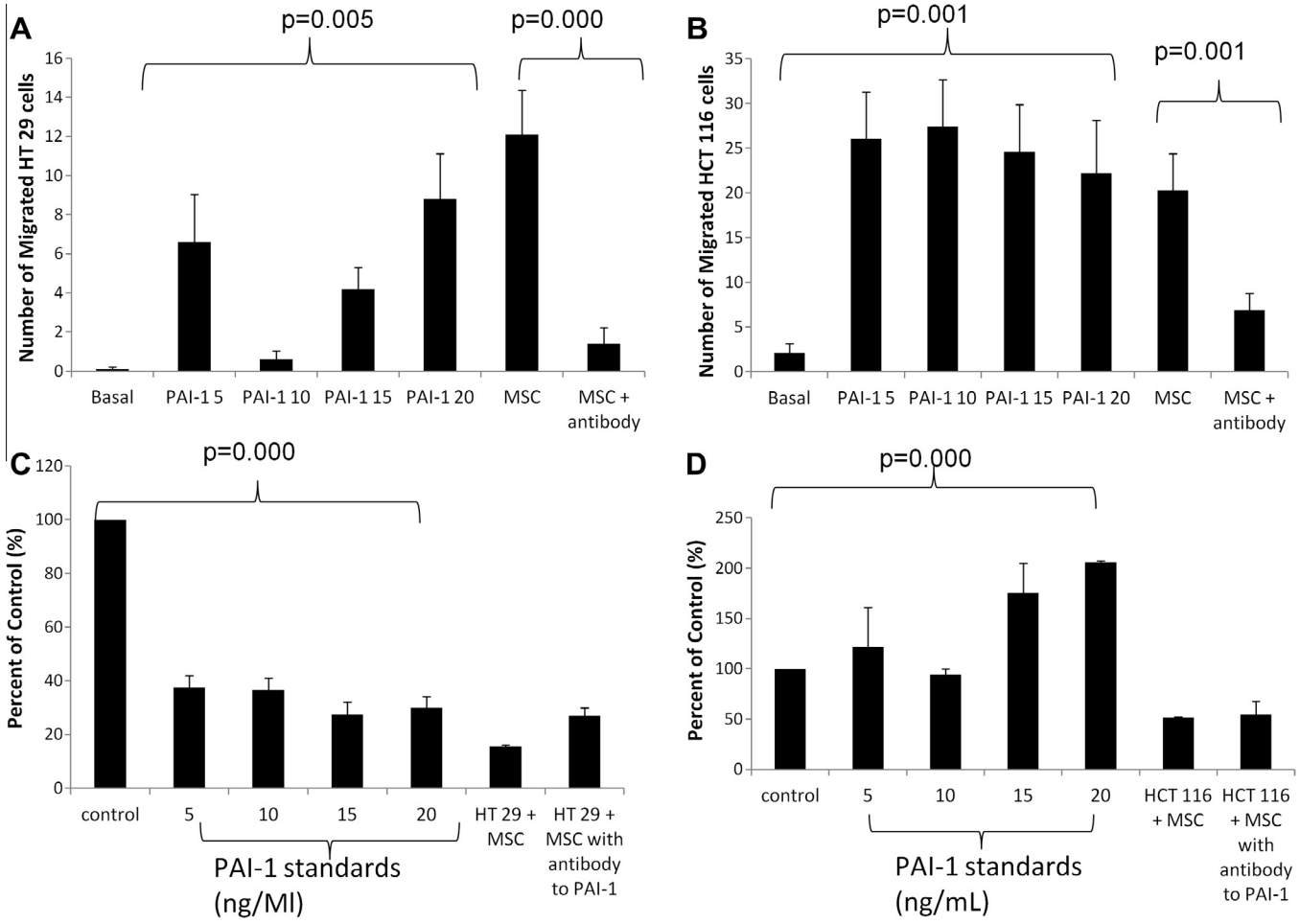

Fig. 4. Effect of PAI-1 on colon cancer cell (CCC) migration and proliferation. Effect of basal media (control), PAI-1 standards (5–20 ng/mL), MSCs and MSCs in the presence of an antibody to PAI-1 (10 ng/mL) on migration of HT29 (A) and HCT-116. (B) cells The effect of these conditions on proliferation of HT29. (C) and HCT116. (D) cells was also determined.

distinct changes in PAI-1 secretion were observed. Both colorectal cancer cell lines were in the presence of higher levels of PAI-1 when MSCs were present (HT29 + MSC: mean 10.43 ng/mL, HCT-116 + MSC mean: 5.79 ng/mL).

3.4. Cell migration

To determine the functional effect of this MSC-secreted PAI-1 on colorectal cancer cells, migration and proliferation in response to the cytokine was examined (Fig. 4). Both HCT-116 and HT29 cells migrated in significant numbers in response to recombinant standards of PAI-1 (5, 10, 15 and 20 ng/mL, Fig. 4). Significant migration was also observed in response to all factors secreted by MSCs seeded in the well below. In the case of HT29, significant blocking of migration (88% inhibition, $p = 0.000$) was observed in the presence of MSCs with an antibody to PAI-1 (10 ng/mL) (Fig 4A). In contrast to HT29 migration, HCT-116 cells exhibited a sustained migratory response to all concentrations of PAI-1 standard

(Fig. 4B). Like HT29 cells, significant migration of HCT-116 cells was also observed in response to all factors secreted by MSCs, with significant blocking of migration (67% inhibition, $p = 0.001$) in the presence of MSCs with an antibody to PAI-1 (10 ng/mL) (Fig 4B).

3.5. Cell proliferation

Recombinant standards of PAI-1 (5–20 ng/mL) exerted an anti-proliferative effect on HT29 cells (60–65% inhibition, Fig. 4C). A decrease in proliferation was also observed in the presence of all factors secreted by MSCs (80% inhibition). Addition of an antibody to PAI-1 (10 ng/mL) abrogated this effect (10% blocking, Fig. 4C), further highlighting an anti-proliferative role for PAI-1 in this setting. In contrast to HT29 cells, no effect on proliferation of HCT-116 cells was observed with concentrations of 5 or 10 ng/mL of PAI-1 recombinant standards. Interestingly, there was a significant increase in proliferation in the presence of higher concentrations of PAI-1 standard (15–20 ng/mL, 75–100% increase respectively, $p = 0.000$, Fig. 4D). All factors secreted by MSCs had an anti-proliferative effect (50% decrease). No blocking of this effect was observed in the presence of an antibody to PAI-1 suggesting the involvement of other secreted factors.

4. Discussion

It is now well established that MSCs migrate to and engraft at the site of many tumour types, including colorectal tumours[6], with conflicting reports on a pro- or anti-tumorigenic role [22]. Due to the remarkable tumour-specific homing capacity of MSCs, they may play a role as tumour targeted delivery agents [22]. Conversely, recent co-injection studies have reported that MSCs promote tumour growth and metastatic potential in colon cancer. However, the majority of studies employed an excess of MSCs to CCCs and are therefore an uncertain reflection of the physiological environment [9]. Shinagawa et al. [6] observed that, following systemic injection via the tail vein, MSCs were functionally incorporated into the colorectal cancer tumour stroma where they displayed morphology and distribution similar to those of carcinoma-associated fibroblasts indicating a potential role in promoting tumorigenesis.

While there has been a wealth of studies in other cancers regarding MSC-epithelial cell interactions, in colon cancer there has been only one recently published report. DeBoeck et al. [8] investigated the role of neuregulin-1 (tNRG1) and human epithelial growth factor regulator (HER), and reported that paracrine NRG1/HER3 signals initiated by MSCs promote CRC progression. In the present study, in vitro interactions between MSCs and CCC and potential mediators were further explored. Interestingly, while CCCs represented the principal source of MIF, MSCs were found to secrete highest levels of PAI-1, a factor strongly linked with colon cancer [16]. This was confirmed through gene expression analysis, with MSCs expressing PAI-1 77-fold higher than CCC lines. It is noteworthy that a previous study has shown by immunofluoresence that myofibroblasts at the leading edge of colorectal tmours express PAI-1 [23]. Also, messenger RNA for PAI-1 has been localised by in situ hybridization to endothelial cells within colon tumours [24]. It is interesting to note that MSCs have been reported to differentiate into endothelial cells and myofibroblasts[25,26]. The results reported herein also demonstrate through 3D culture, the significant impact of even a relatively small number of MSCs on the micro-environment of CCC. Furthermore, functional analysis revealed that this MSC-secreted PAI-1 has a significant pro-migratory effect on CCCs. In contrast, proliferation was affected in a cell line dependant manner.

This study shows that as a component of the microenvironment, Mesenchymal Stem Cells may represent a significant source of PAI-1 in colon tumours. Given that high serum and tissue levels of PAI-1 in colon cancer patients have been shown to correlate with poor prognosis [15], this may have significant clinical implications in development of novel therapies [6]. The current study demonstrates that as a component of the colonic tumour environment, MSCs have a significant functional effect and may represent key therapeutic targets.

Conflict of interest

The authors declare that they have no competing interests.

Author's contribution

NMH performed the experiments and wrote the manuscript, MRJ was involved in study design and drafting of the final manuscript, JMM, FPB and TO'B provided Mesenchymal Stem Cells and technical support, MJK was involved in study design, RMD conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References

[1] J.B. O'Connell, M.A. Maggard, C.Y. Ko, Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging, J. Natl. Cancer Inst. 96 (2004) 1420–1425.
[2] H. Nakagawa, S. Liyanarachchi, R.V. Davuluri, H. Auer, E.W. Martin Jr., A. de la Chapelle, W.L. Frankel, Role of cancer-associated stromal fibroblasts in metastatic colon cancer to the liver and their expression profiles, Oncogene 23 (2004) 7366–7377.
[3] S. Gout, J. Huot, Role of cancer microenvironment in metastasis: focus on colon cancer, Cancer Microenviron. 1 (2008) 69–83.
[4] W.E. Mesker, J.M. Junggeburt, K. Szuhai, P. de Heer, H. Morreau, H.J. Tanke, R.A. Tollenaar, The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage, Cell. Oncol. 29 (2007) 387–398.
[5] 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.
[6] K. Shinagawa, Y. Kitadai, M. Tanaka, T. Sumida, M. Kodama, Y. Higashi, S. Tanaka, W. Yasui, K. Chayama, Mesenchymal stem cells enhance growth and metastasis of colon cancer, Int. J. Cancer 127 (2010) 2323–2333.
[7] Y. Liu, Z.P. Han, S.S. Zhang, Y.Y. Jing, X.X. Bu, C.Y. Wang, K. Sun, G.C. Jiang, X. Zhao, R. Li, L. Gao, Q.D. Zhao, M.C. Wu, L.X. Wei, Effects of inflammatory factors on mesenchymal stem cells and their role in the promotion of tumor angiogenesis in colon cancer, J. Biol. Chem. 286 (2011) 25007–25015.
[8] A. De Boeck, P. Pauwels, K. Hensen, J.L. Rummens, W. Westbroek, A. Hendrix, D. Maynard, H. Denys, K. Lambein, G. Braems, C. Gespach, M. Bracke, O.D. Wever, Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression through paracrine neuregulin 1/HER3 signalling, Gut 62 (2013) 550–560.
[9] N.M. Hogan, R.M. Dwyer, M.R. Joyce, M.J. Kerin, Mesenchymal stem cells in the colorectal tumor microenvironment: recent progress and implications, Int. J. Cancer 131 (2012) 1–7.
[10] A. Alphonso, S.K. Alahari, Stromal cells and integrins: conforming to the needs of the tumor microenvironment, Neoplasia 11 (2009) 1264–1271.
[11] T. Calandra, T. Roger, Macrophage migration inhibitory factor: a regulator of innate immunity, Nat. Rev. Immunol. 3 (2003) 791–800.
[12] H. Conroy, L. Mawhinney, S.C. Donnelly, Inflammation and cancer: macrophage migration inhibitory factor (MIF)–the potential missing link, QJM 103 (2010) 831–836.
[13] R.A. Mitchell, R. Bucala, Tumor growth-promoting properties of macrophage migration inhibitory factor (MIF), Semin Cancer Biol. 10 (2000) 359–366.
[14] X.X. He, K. Chen, J. Yang, X.Y. Li, H.Y. Gan, C.Y. Liu, T.R. Coleman, Y. Al-Abed, Macrophage migration inhibitory factor promotes colorectal cancer, Mol. Med. 15 (2009) 1–10.
[15] K. Dano, N. Behrendt, G. Hoyer-Hansen, M. Johnsen, L.R. Lund, M. Ploug, J. Romer, Plasminogen activation and cancer, Thromb. Haemost. 93 (2005) 676–681.
[16] D.H. Berger, Plasmin/plasminogen system in colorectal cancer, World J. Surg. 26 (2002) 767–771.
[17] R.D. Balsara, V.A. Ploplis, Plasminogen activator inhibitor-1: the double-edged sword in apoptosis, Thromb. Haemost. 100 (2008) 1029–1036.
[18] J.H. Hwang, S.S. Shim, O.S. Seok, H.Y. Lee, S.K. Woo, B.H. Kim, H.R. Song, J.K. Lee, Y.K. Park, Comparison of cytokine expression in mesenchymal stem cells from human placenta, cord blood, and bone marrow, J. Korean Med. Sci. 24 (2009) 547–554.

[19] F.P. Barry, J.M. Murphy, Mesenchymal stem cells: clinical applications and biological characterization, Int. J. Biochem. Cell Biol. 36 (2004) 568–584.
[20] F.T. Martin, R.M. Dwyer, J. Kelly, S. Khan, J.M. Murphy, C. Curran, N. Miller, E. Hennessy, P. Dockery, F.P. Barry, T. O'Brien, M.J. Kerin, Potential role of mesenchymal stem cells MSCs) in the breast tumour microenvironment: stimulation of epithelial to mesenchymal transition (EMT), Breast Cancer Res. Treat. 124 (2010) 317–326.
[21] 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.
[22] R.M. Dwyer, M.J. Kerin, Mesenchymal stem cells and cancer: tumor-specific delivery vehicles or therapeutic targets?, Hum Gene Ther. 21 (2010) 1506–1512.
[23] M. Illemann, U. Hansen, H.J. Nielsen, P.A. Andreasen, G. Hoyer-Hansen, L.R. Lund, K. Dano, B.S. Nielsen, Leading-edge myofibroblasts in human colon cancer express plasminogen activator inhibitor-1, Am. J. Clin. Pathol. 122 (2004) 256–265.
[24] C. Pyke, P. Kristensen, E. Ralfkiaer, J. Eriksen, K. Dano, The plasminogen activation system in human colon cancer: messenger RNA for the inhibitor PAI-1 is located in endothelial cells in the tumor stroma, Cancer Res. 51 (1991) 4067–4071.
[25] J. Oswald, S. Boxberger, B. Jorgensen, S. Feldmann, G. Ehninger, M. Bornhauser, C. Werner, Mesenchymal stem cells can be differentiated into endothelial cells in vitro, Stem Cells 22 (2004) 377–384.
[26] R.M. Baertschiger, V. Serre-Beinier, P. Morel, D. Bosco, M. Peyrou, S. Clement, A. Sgroi, A. Kaelin, L.H. Buhler, C. Gonelle-Gispert, Fibrogenic potential of human multipotent mesenchymal stromal cells in injured liver, PLoS One 4 (2009) e6657.