

Ovarian cancer-derived ascitic fluids induce a senescence-dependent pro-cancerogenic phenotype in normal peritoneal mesothelial cells

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Abstract

Purpose After the seeding ovarian cancer cells into the peritoneal cavity, ascitic fluid creates a microenvironment in which these cells can survive and disseminate. The exact nature of the interactions between malignant ascitic fluids and peritoneal mesothelial cells (HPMCs) in ovarian cancer progression has so far remained elusive. Here we assessed whether malignant ascitic fluids may promote the senescence of

HPMCs and, by doing so, enhance the acquisition of their pro-cancerogenic phenotype.

Methods Primary omentum-derived HPMCs, ovarian cancer-derived cell lines (A2780, OVCAR-3, SKOV-3), malignant ascitic fluids and benign ascitic fluids from non-cancerous patients were used in this study. Ovarian cancer cell proliferation, as well as HPMC proliferation and senescence, were determined using flow cytometry and β -galactosidase assays, respectively. Ovarian cancer cell migration was quantified using a Transwell assay. The concentrations of soluble agents in ascitic fluids, conditioned media and cell lysates were measured using DuoSet® Immunoassay Development kits.

Results We found that HPMCs, when exposed to malignant ascitic fluids, exhibited decreased proliferation and increased senescence rates. The malignant ascitic fluids were found to contain elevated levels of HGF, TGF- β 1 and GRO-1, of which HGF and GRO-1 were able to induce senescence in HPMCs. We also found that HPMCs subjected to malignant ascitic fluids or exogenously added HGF and GRO-1 stimulated ovarian cancer cell progression, which was manifested by an increased production of HA (adhesion), uPA (proliferation), IL-8 and MCP-1 (migration).

Conclusion Our results indicate that malignant ascitic fluids may contribute to ovarian cancer progression by accelerating the senescence of HPMCs.

Keywords Cellular senescence · Malignant ascites · Ovarian cancer · Peritoneal mesothelium

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1 Introduction

Ovarian cancer is one of the leading causes of cancer-related death in women worldwide [1–3]. A unique feature of ovarian

cancer is its predilection for the peritoneal cavity as a place of metastasis. Seeding of the peritoneum with tumor cells is often associated with an accumulation of ascitic fluid [4]. An excess of malignant ascitic fluid is usually present in advanced ovarian cancer, although this may also be the case in earlier stages of the disease [5]. There is general agreement that malignant ascitic fluids create a specific, permissive microenvironment in the peritoneal cavity in which ovarian cancer cells can survive and disseminate [6]. Specifically, ascitic fluid is rich in soluble agents that support angiogenesis and the proliferation of ovarian cancer cells, including angiogenin, vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and the chemokines MCP-1, GRO-1 and IL-8 [7]. Moreover, malignant ascitic fluids are capable of suppressing peritoneal inflammatory reactions, including the inhibition of T-cell receptor-induced nuclear factor-kappa B (NF- κ B) and the nuclear factor of activated T-cell (NFAT) signaling in tumor-associated T-cells [8]. Last but not least, ascitic fluids can interact with normal peritoneal mesothelial cells (HPMCs), thereby promoting their proliferation and migration, and inhibiting HPMC-dependent apoptosis of cancer cells [9, 10].

HPMCs in the peritoneal cavity are believed to actively support various aspects of cancer cell progression, including adhesion and proliferation [11, 12]. Interestingly, it has been found that the pro-cancerogenic activities of HPMC, including their pro-adhesive and pro-angiogenic activities, are enhanced when they approach the end of their replicative lifespan and become senescent [13, 14]. In fact, there is mounting evidence indicating that ovarian cancer cells that are accompanied by senescent HPMCs progress more rapidly than those accompanied by young HPMCs, either *in vitro* or *in vivo* in a mouse peritoneal cavity [14, 15].

Based on increasing knowledge on the role of senescent HPMCs in cancer cell progression, as well as on the possibility that the above-mentioned increase in HPMC proliferation in response to malignant ascitic fluids [9] may lead to an accelerated exhaustion of the finite number of achievable cell divisions (i.e., the Hayflick limit) and, thus, to an accelerated entry into senescence, we hypothesized that malignant ascitic fluids may induce cellular senescence in HPMCs which, in turn, may lead to the induction of a senescence-specific, pro-cancerogenic phenotype in these cells.

2 Materials and methods

2.1 Materials

Unless otherwise stated, all chemicals and disposables were from Sigma (St. Louis, MO, USA). Exogenous proteins and hyaluronic acid (HA) were obtained from R&D Systems (Abingdon, UK).

2.2 Cell cultures

Human peritoneal mesothelial cells (HPMCs) were isolated from omentum samples obtained from 8 patients (36–40 years old) undergoing elective abdominal surgery (institutional consent number 187/14) as described in detail elsewhere [16]. The reasons for abdominal surgery included aortic aneurysm (3), hernia (3) and bowel obstruction (2). Briefly, the tissue samples were incubated in a solution of 0.05 % trypsin and 0.02 % EDTA for 20 min at 37 °C while gentle shaking. Subsequently, the digestive solution was removed and the isolated cells were suspended in medium M199 with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μ g/ml), hydrocortisone (0.4 μ g/ml) and 10 % fetal bovine serum (FBS). The cells were maintained at 37 °C in a humidified atmosphere containing 95 % air and 5 % CO₂. The cells were classified as pure mesothelial by their typical cobblestone appearance at confluency and their uniform positive staining for cytokeratins and HBME-1 antigen. Only primary HPMC cultures from the 1st passage (corresponding to ~5 % of their replicative lifespan) without any contamination with stromal cells were used in the experiments.

The ovarian cancer-derived cell lines A2780 and SKOV-3 were purchased from the ECCC (Porton Down, UK) and propagated in RPMI-1640 medium supplemented with L-glutamine (2 mmol/L), penicillin (100 U/ml), streptomycin (100 g/ml) and 10 % FBS. The ovarian cancer-derived OVCAR-3 cells were purchased from the ATCC (Rockville, MD, USA) and grown in RPMI-1640 medium supplemented with L-glutamine (2 mmol/L), HEPES (10 mmol/L), sodium pyruvate (1 mmol/L), glucose (4500 mg/L), insulin (0,01 mg/ml) and 20 % FBS.

2.3 Collection and preparation of ascitic fluids

Malignant ascitic fluids were obtained at the time of cytoreductive surgery from 8 patients (68–78 years old) with serous ovarian carcinomas at stages IIIC and IV. The histopathology, grade and stage of the tumors were assigned in accordance with the criteria of the International Federation of Gynecology and Obstetrics. The patients did not receive chemotherapy prior to surgery. Control, benign ascitic fluids were obtained from 8 age-matched patients undergoing abdominal surgery due to the presence of non-cancerous lesions, i.e., cystadenoma mucinosum multiloculare (institutional consent number 543/14). Upon collection under sterile conditions, the fluids were centrifuged at 2500 rpm for 5 min after which cell-free supernatants were stored in aliquots at –20 °C until use.

2.4 HPMC proliferation and senescence assays

The proliferation rates of HPMCs exposed to 10 % ascitic fluid (for 72 h) were quantified through flow cytometric

analyses of the percentages of dividing, i.e., DNA-synthesizing cells in the S-phase of the cell cycle, as reported before [17]. The quantification of senescent cells was performed through the measurement of senescence-associated β -galactosidase (SA- β -Gal) activity, as reported before [18]. In addition, the activity of SA- β -Gal in HPMCs was examined upon exposure (72 h) to exogenous, recombinant GRO-1, HGF and TGF- β 1 (separately or in combination) at concentrations corresponding to the average levels of the cytokines in malignant ascitic fluids.

2.5 Immunoassays

The concentrations of soluble agents in ascitic fluids, conditioned media and cell lysates were determined using appropriate DuoSet® Immunoassay Development kits (R&D Systems), following the manufacturer's instructions.

2.6 Cancer cell adhesion, proliferation and migration assays

The adhesion of ovarian cancer cells probed with a fluorescence dye (calcein AM) to HPMCs was examined according to a method reported before [14]. The proliferation of ovarian cancer cells exposed to conditioned media (CM) from HPMCs was examined using flow cytometry (percentage S-phase cells) as reported before [17]. Ovarian cancer cell migration towards a chemotactic gradient generated by HPMC-derived CM was quantified using Transwell inserts (Costar, Inc., NY, USA), as reported before [19]. In addition, ovarian cancer cell proliferation and migration were assessed in response to CMs generated by HPMCs exposed to exogenous, recombinant forms of human GRO-1 and HGF, whereas ovarian cancer cell adhesion to HPMCs subjected to these agents was assessed directly. The concentrations of GRO-1 and HGF used corresponded to their levels in malignant ascitic fluids. In addition, ovarian cancer cell proliferation and migration were tested in the presence of recombinant uPA and VEGF (proliferation) and IL-8 and MCP-1 (migration). To assess cell adhesion, HPMCs were incubated with exogenous HA to generate a coat encompassing the cells. After this, the medium was gently removed and ovarian cancer cells were added to determine the effectiveness of their attachment. Exogenous uPA, VEGF, IL-8, MCP-1 and HA were used in concentrations corresponding to those in HPMC conditioned media upon exposure to malignant ascitic fluids.

2.7 Collection of conditioned media

HPMCs (4×10^5) were exposed to ascitic fluids (10 %) for 72 h, washed carefully with PBS and incubated with serum-free medium (M199) for 48 h to generate conditioned medium (CM). The CM were cleared by centrifugation, filtered

through a 0.2 μ m pore size filter and stored at -80 °C until use.

2.8 Statistic analyses

Statistic analyses were performed using GraphPad Prism™ v.5.00 software (GraphPad Software, San Diego, USA). Means were compared using the Wilcoxon matched-pairs test. Results were expressed as means \pm SEM. Differences with a p -value < 0.05 were considered to be statistically significant.

3 Results

3.1 Malignant ascitic fluids affect HPMC proliferation and senescence

HPMCs obtained from the first passage (see materials and methods) were exposed to 10 % malignant and 10 % control (from patients with benign tumors) ascitic fluids for 72 h, after which their proliferation and senescence rates were evaluated by flow cytometry and SA- β -Gal assays, respectively. By doing so, we found that the percentage of cells in the S-phase of the cell cycle in cultures exposed to malignant ascitic fluids was decreased by 40 ± 2 % ($p < 0.01$) compared to their counterparts subjected to the control ascitic fluids. This effect coincided with an increased activity of SA- β -Gal, an indicator of cellular senescence, by 25 ± 5 % ($p < 0.03$) compared to the control group (Fig. 1). After re-analyzing these data using the results of our previous study on the relationship between SA- β -Gal activity and the size of the SA- β -Gal-positive cell population [18], we found that the background level of SA- β -Gal in HPMCs maintained in standard medium (not shown)

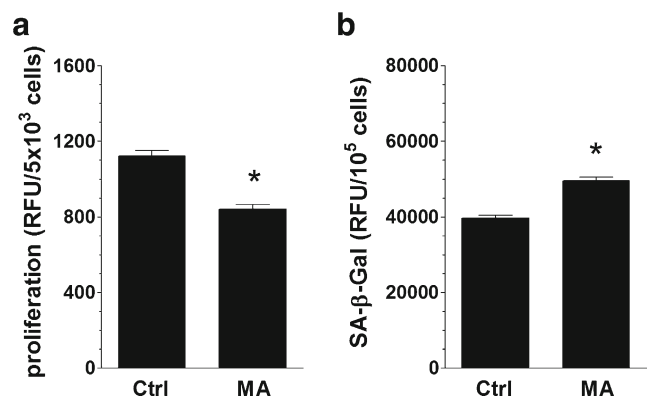


Fig. 1 Effect of malignant ascitic fluids on the proliferation (a) and senescence (b) of HPMCs. Proliferation and senescence (SA- β -Gal activity) rates of HPMCs exposed to malignant ascitic fluids (MA) compared to those of HPMCs exposed to control, benign fluids (Ctrl). The asterisks indicate significant differences compared to the control group. The HPMCs were derived from 8 different donors, and the malignant and benign fluids were derived from 8 different patients each. RFU – relative fluorescence units

corresponded to $\sim 8 \pm 2\%$ of the senescent cells. At the same time, we found that cultures exposed to benign ascitic fluids contained $\sim 10 \pm 4\%$ prematurely senescent cells (no significant difference), whereas in cultures exposed to malignant ascitic fluids the subset of senescent cells increased to $34 \pm 4\%$ ($p < 0.01$).

3.2 The senescence-promoting activity of malignant ascitic fluids is associated with an increased production and activity of GRO-1 and HGF

In order to identify mediator(s) of the senescence-promoting activity of malignant ascitic fluids, malignant and benign ascitic fluids were assessed for their concentrations of 11 selected soluble factors (IL-1 β , IL-6, IL-8, GRO-1, HGF, MCP-1, PAI-1, SDF-1, TGF- β 1, TNF α , uPA) that are known to be constitutively present in malignant ascitic fluids and that, according to literature data and our own pilot experiments, might be capable of inducing senescence in normal cells. We found that the concentrations of 3 of these factors, i.e. GRO-1, HGF and TGF- β 1, differed in malignant versus benign ascitic fluids (Table 1). The levels of these factors were uniformly higher in the malignant ascitic fluids, i.e., by $423 \pm 12\%$ ($p < 0.01$), by $271 \pm 22\%$ ($p < 0.01$) and by $39 \pm 3\%$ ($p < 0.05$) for GRO-1, HGF and TGF- β 1, respectively (Fig. 2a–c).

In order to determine which of these factors was responsible for the increased fraction of senescent HPMC cells in cultures treated with malignant ascitic fluids, exogenous recombinant forms of these factors were used at concentrations corresponding to those in malignant ascitic fluids, after which the activity of SA- β -Gal was measured. We found that the activity of SA- β -Gal in the HPMCs was increased when they

were exposed for 72 h to exogenous GRO-1 ($43 \pm 6\%$, $p < 0.04$) and HGF ($29 \pm 3\%$, $p < 0.04$). After exposure to TGF- β 1, however, the activity of SA- β -Gal remained unchanged (Fig. 2d). Additional co-incubation experiments did not reveal any synergistic activities of HGF, GRO-1 and TGF- β 1, and excluded a potentiating effect of TGF- β 1 towards the activities exerted by HGF and GRO-1 (data not shown).

3.3 Malignant ascitic fluid-induced senescent HPMCs enhance ovarian cancer cell progression

The adhesion of three representative serous ovarian cancer-derived cell lines (A2780, OVCAR-3, SKOV-3) to HPMCs, as well as their proliferation and migration in response to HPMC conditioned medium (CM), were assessed in order to address the question whether malignant ascitic fluid-induced senescent HPMCs may exert ovarian cancer-promoting activities. We found that HPMCs exposed to malignant ascitic fluids stimulated the adhesion process compared to those exposed to control benign ascitic fluids, in case of A2780 cells by $30 \pm 2\%$ ($p < 0.03$) and SKOV-3 cells by $47 \pm 6\%$ ($p < 0.03$), but not in OVCAR-3 cells (Fig. 3a). The proliferation rate of all three ovarian cancer-derived cell lines exposed to CM from HPMCs treated with malignant ascitic fluid was found to be increased by $20 \pm 3\%$ ($p < 0.05$) for A2780 cells, by $24 \pm 1\%$ ($p < 0.04$) for OVCAR-3 cells and by $22 \pm 3\%$ ($p < 0.05$) for SKOV-3 cells compared to the control group (Fig. 3b). The migration of ovarian cancer cells towards a chemotactic gradient established by CM produced by HPMCs subjected to malignant ascitic fluid was also uniformly increased, i.e., by $13 \pm 3\%$ ($p < 0.05$) for A2780 cells, by $21 \pm 5\%$ ($p < 0.04$) for OVCAR-3 cells and by $9 \pm 2\%$ ($p < 0.05$) for SKOV-3 cells, respectively (Fig. 3c).

Next, we set out to test the effectiveness of ovarian cancer cell progression (proliferation, migration) in the presence of CM generated by HPMCs exposed to exogenous GRO-1 and HGF (at the same concentrations at which they were capable of inducing SA- β -Gal), or in direct physical contact with GRO-1/HGF-treated HPMCs (adhesion). By doing so, we found that HPMCs exposed to both GRO-1 and HGF significantly enhanced all three characteristics of ovarian cancer cell progression, and that the effects obtained were consistent throughout the cell lines tested (Fig. 4a–c).

3.4 Senescent HPMC-enhanced ovarian cancer cell progression is associated with an increased production of pro-cancerous factors

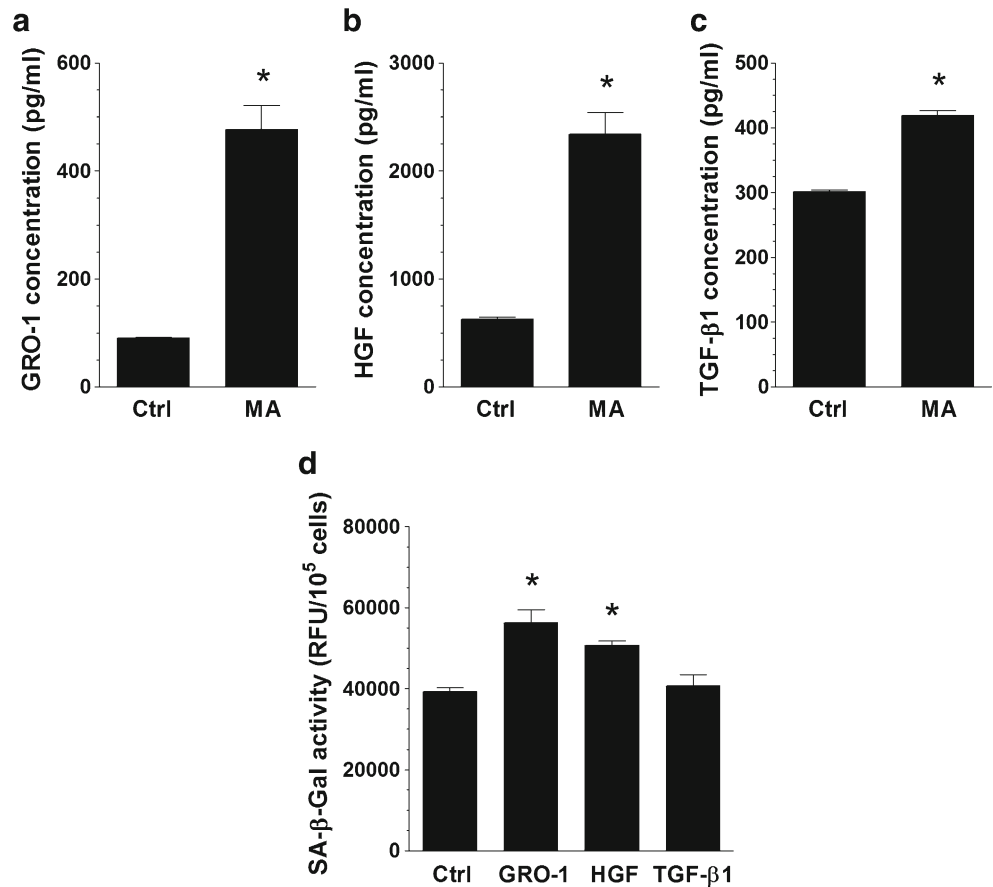
The production of 10 selected mediators of ovarian cancer cell progression, i.e., $\alpha 5\beta 1$ integrin and HA (adhesion [20]),

Table 1 Concentration of soluble mediators of cancer progression in benign ascitic fluids and malignant ascitic fluids from patients with serous ovarian cancer

Soluble agent (pg/ml)	Benign ascites	Malignant ascites	<i>P</i> -value
IL-1 β	n.d.	n.d.	n.s.
IL-6	815 \pm 13	922 \pm 12	n.s.
IL-8	450 \pm 72	536 \pm 82	n.s.
GRO-1	91 \pm 1	476 \pm 44	$p < 0.01$
HGF	630 \pm 14	2340 \pm 200	$p < 0.01$
MCP-1	325 \pm 5	330 \pm 25	n.s.
PAI-1	634 \pm 67	751 \pm 44	n.s.
SDF-1	594 \pm 54	611 \pm 95	n.s.
TGF- β 1	301 \pm 2	419 \pm 7	$p < 0.05$
TNF α	n.d.	n.d.	n.s.
uPA	466 \pm 81	633 \pm 78	n.s.

Results are expressed as means \pm SEM. n.d. – not detectable, n.s. – not significant

Fig. 2 Identification of senescence-promoting agents in malignant ascitic fluids. Comparison of malignant ascitic (MA) and control benign ascitic (Ctrl) fluids with respect to the concentration of GRO-1 (a), HGF (b) and TGF- β 1 (c). Activity of SA- β -Gal (versus non-treated cells; Ctrl) in HPMCs exposed to exogenous, recombinant forms of GRO-1, HGF and TGF- β 1 (d). The asterisks indicate significant differences compared to the control group. The HPMCs were derived from 8 different donors, and the malignant and benign fluids were derived from 8 different patients each



GRO-1, IL-6, uPA and VEGF (proliferation [21–24]) and HGF, IL-8, PAI-1 and MCP-1 (migration [10, 24–26]) by malignant ascitic fluid-induced senescent HPMCs was examined in order to reveal which of these factors might be responsible for their ovarian cancer-promoting activity. We found that HPMCs subjected to malignant ascitic fluids produced increased amounts of HA, uPA, VEGF, IL-8 and MCP-1 compared to their counterparts exposed to benign ascitic fluids. We also found that the secretion of the other factors remained unchanged (Fig. 5a).

Next, exogenous forms of HA, uPA, VEGF, IL-8 and MCP-1 were used to verify whether the altered production of these factors plays a genuine role in the progression of ovarian cancer cells. In case of HA, we found that an additional coat covering the surface of HPMCs increased the fraction of ovarian cancer cells that adhered to HPMCs, i.e., by $32 \pm 4\%$ ($p < 0.02$) for A2780 cells, by $23 \pm 3\%$ ($p < 0.05$) for OVCAR-3 cells and by $27 \pm 4\%$ ($p < 0.04$) for SKOV-3 cells (Fig. 5b). In addition, we found that exogenous uPA (but not VEGF), used at a dose corresponding to that in the CM from

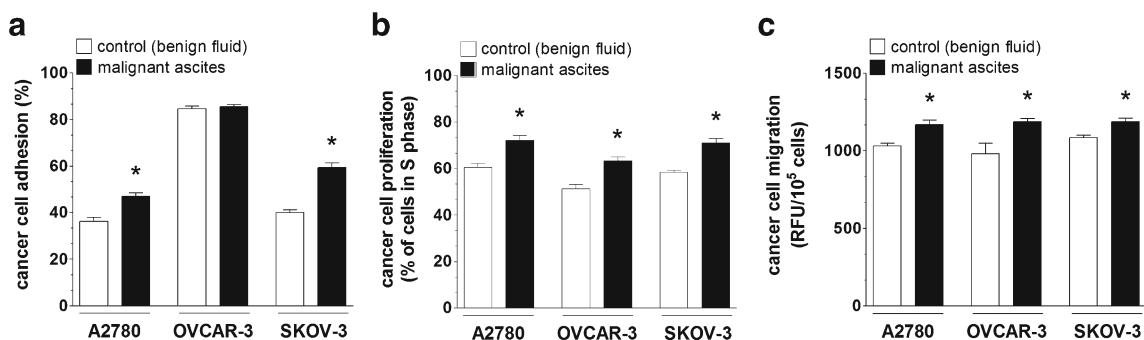


Fig. 3 Effect of malignant ascitic fluid-induced senescent HPMCs on the adhesion (a), proliferation (b) and migration (c) of ovarian cancer cells. The asterisks indicate significant differences compared to the control

group. The HPMCs were derived from 8 different donors, and the malignant and benign fluids were derived from 8 different patients each. RFU – relative fluorescence units

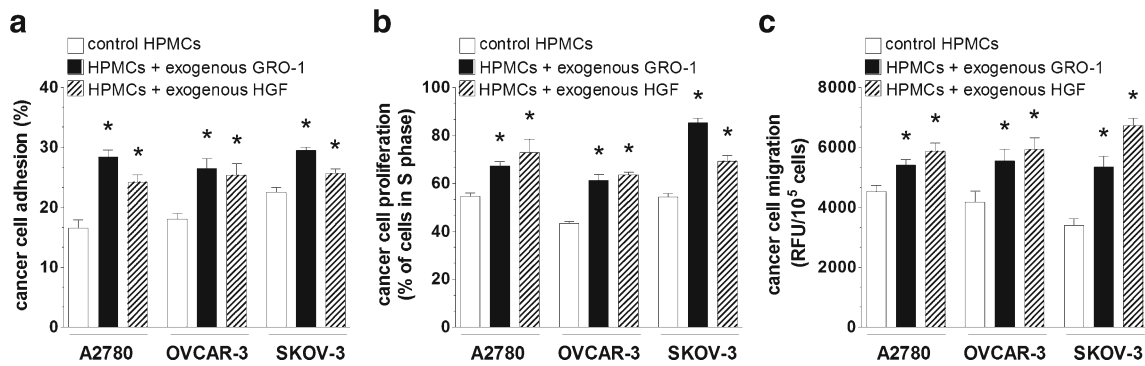


Fig. 4 Effect of HPMCs exposed to exogenous, recombinant forms of GRO-1 and HGF on the adhesion (a), proliferation (b) and migration (c) of ovarian cancer cells. The asterisks indicate significant differences as

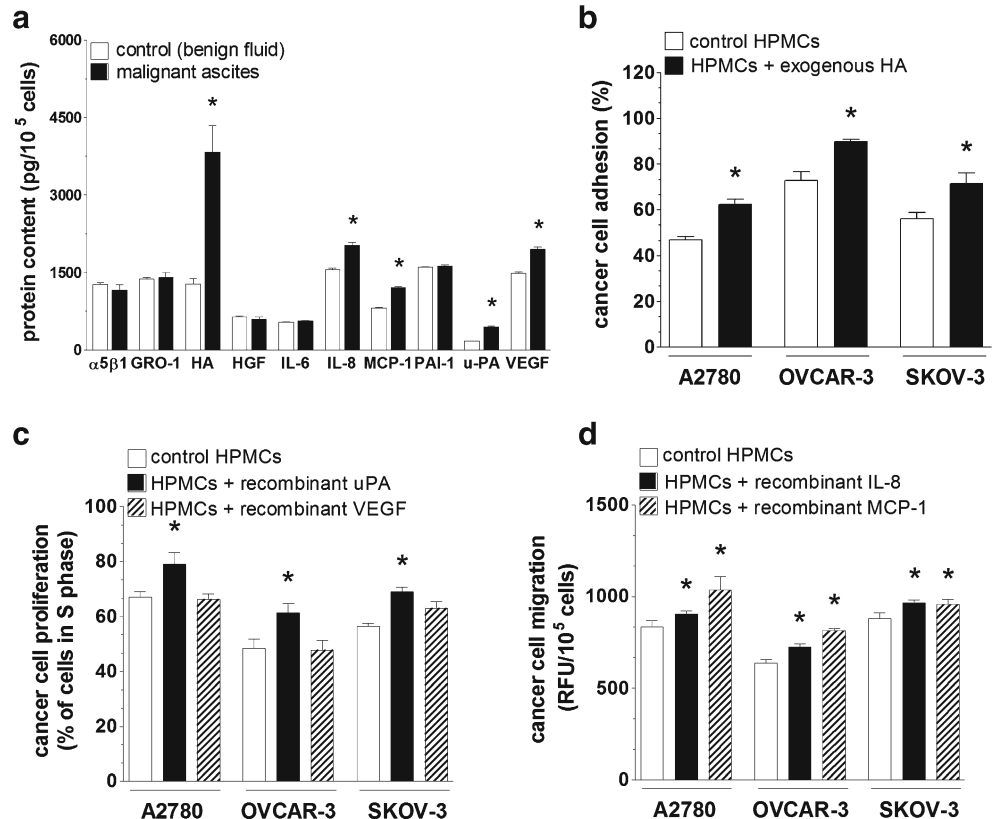
compared to the control group (cells not treated with GRO-1 or HGF). The HPMCs were derived from 8 different donors. The cancer cells were used in duplicates. RFU – relative fluorescence units

HPMCs subjected to malignant ascitic fluid, stimulated the proliferation of A2780 cells by $18 \pm 1 \%$ ($p < 0.05$), of OVCAR-3 cells by $25 \pm 3 \%$ ($p < 0.03$) and of SKOV-3 cells by $21 \pm 3 \%$ ($p < 0.04$) (Fig. 5c). With respect to ovarian cancer cell migration, we found that both IL-8 and MCP-1 enhanced this process (Fig. 5d). IL-8 stimulated the migration by $8 \pm 2 \%$ ($p < 0.05$) in A2780 cells, by $13 \pm 2 \%$ ($p < 0.05$) in OVCAR-3 cells and by $10 \pm 3 \%$ ($p < 0.05$) in SKOV-3 cells. MCP-1 stimulated the migration by $24 \pm 3 \%$ ($p < 0.04$) in A2780 cells, by $28 \pm 1 \%$ ($p < 0.04$) in OVCAR-3 cells and by $9 \pm 2 \%$ ($p < 0.05$) in SKOV-3 cells.

4 Discussion

Although the role of malignant ascitic fluid in the progression of ovarian cancer is well established, the mechanisms by which this fluid exerts its adverse, cancer-promoting effects has so far remained unclear [27]. One of the less understood aspects of the cancer-promoting effect of malignant ascitic fluid is its interaction with peritoneal mesothelial cells (HPMCs), which have been recognized as being critically engaged in the intraperitoneal spread of ovarian cancer [11, 12]. Here, we aimed at addressing the question whether

Fig. 5 Identification of mediators of increased cancer cell progression, generated by HPMCs exposed to malignant ascitic fluids. Production of proteins involved in cancer cell adhesion, proliferation and migration by HPMCs exposed to malignant ascites (a). Effect of exogenous HA on the efficiency of ovarian cancer cell adhesion to HPMCs (b). Effect of recombinant uPA and VEGF on the proliferation of ovarian cancer cells (c). Effect of recombinant IL-8 and MCP-1 on the migration of ovarian cancer cells. The asterisks indicate significant differences compared to the control group. The HPMCs were derived from 8 different donors, and the malignant and benign fluids were derived from 8 different patients each. RFU – relative fluorescence units



malignant ascitic fluids can modify the senescence rate of HPMCs and, if so, whether these modified HPMCs can support ovarian cancer cell progression. As of yet, the effect of malignant ascitic fluids on the senescence of normal HPMCs has not been studied, despite accumulating evidence that points at their vital, stimulatory role in the development of intraperitoneal cancer metastases [15, 28].

In this report we compared the effect of malignant ascitic fluids derived from patients with serous ovarian carcinoma, which is the most frequent type of ovarian cancer [29], with that of benign ascitic fluids derived from patients undergoing abdominal surgery for reasons other than cancer [10]. In doing so, we found that malignant ascitic fluids reduce the proliferation and enhance the senescence rates of primary omental HPMCs. Regarding the reduced proliferation, which is in disagreement with previous findings reported by Matte et al. [9], we suspect that the senescence that we observed does not result from exhausting the capacity of HPMCs to divide ('replicative senescence') but, instead, may represent 'stress-induced premature senescence' (SIPS), which is initiated as a rapid response of cells to an environmental insult. This is an intriguing possibility, especially in view of the very recent suggestion of Raghuram and Mishra [30] that SIPS may play an important role in ovarian cancer development.

It is well known that cellular senescence, including SIPS, may be triggered by factors that are usually present in a soluble form in the environment [17]. In order to establish which factors within the malignant ascitic fluids are responsible for their senescence-inducing activity, we found that ascitic fluids obtained from patients with serous ovarian carcinomas exhibited a specific cytokine pattern distinct from those seen in ascitic fluids generated by pseudomyxoma peritonei [31] and malignant mesothelioma [32]. Specifically, we found that the ovarian cancer-derived ascitic fluids exhibited an elevated (compared to benign fluids) concentration of 3 out of 11 factors tested, i.e., GRO-1, HGF and TGF- β 1. Of these three factors, only GRO-1 and HGF appeared to be able to induce SA- β -Gal activity (senescence) in young HPMCs when subjected exogenously to their recombinant forms. This finding was, to some extent, unexpected, because TGF- β 1, which was previously identified as a key mediator of senescence in HPMCs [17], fibroblasts [33] and keratinocytes [34], failed to induce this process when it was used at a dose corresponding to that in malignant ascitic fluids. With respect to GRO-1, our results are in line with those of Yang et al. who found that GRO-1-related signaling may elicit senescence in stromal fibroblasts [35]. With respect to HGF, however, its elevated level and activity have so far been considered to result from cellular senescence rather than to act as a trigger [36]. Taking into account the profound activity of HGF in the course of ovarian cancer development [37], its role as a malignant ascitis-derived senescence-stimulating agent warrants further investigation.

Taking into account the ability of senescent cells to promote cancer cell progression [38], the pro-cancerous potential of malignant ascitic fluid-induced senescent HPMCs, i.e., their effect on ovarian cancer cell adhesion, proliferation and migration, was tested. We found that, by employing three of the most extensively studied serous ovarian cancer-derived cell lines (A2780, OVCAR-3, SKOV-3 [39]), senescent HPMCs enhanced all three characteristics of cancer cell progression tested. Interestingly, we noted that this cancer-promoting activity was related either to direct cell-cell contact or to the activity of soluble factors released by the HPMCs. When the behavior of the ovarian cancer cells was assessed in response to HPMCs subjected to exogenous GRO-1 and HGF, their adhesion, proliferation and migration were enhanced, thus confirming the role of these cytokines as malignant ascitis-derived mediators of HPMC-dependent exacerbation of ovarian cancer cell progression.

Since the pro-cancerogenic action of senescent cells is primarily related to their ability to produce increased amounts of factors capable of promoting cancer cell progression [40], we examined whether senescent HPMCs overproduce such factors. By doing so, we indeed found that these cells produced increased amounts of HA, uPA, VEGF, IL-8 and MCP-1. In order to independently verify the role of these factors in ovarian cancer cell progression, their exogenous recombinant forms were used to evaluate their effects on the respective ovarian cancer progression-related processes. These experiments revealed that the increased adhesion of ovarian cancer cells to modified HPMCs was mediated by HA, that the increased proliferation of these cells was mediated by uPA and that the increased migration of these cells was mediated by IL-8 and MCP-1.

5 Conclusions

Taken together, we here provide evidence for a new mechanism by which malignant ascitic fluids may contribute to the intraperitoneal spread of ovarian cancer cells. This mechanism is based on their ability to induce senescence in HPMCs and to promote a pro-cancerogenic phenotype in these cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study. The experiments were approved by an institutional ethics committee (consent numbers: 187/14 and 543/14).

References

1. Y. Li, K. Wang, Y. Z. Jiang, X. W. Chang, C. F. Dai, J. Zheng, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Inhibits human ovarian cancer cell proliferation. *Cell. Oncol.* **37**, 429–437 (2014)
2. M. Momeni, T. Kalir, S. Farag, L. Chuang, D. Fishman, D. E. Burstein, Expression of H1.5 and PLZF in granulosa cell tumors and normal ovarian tissues: a short report. *Cell. Oncol.* **37**, 229–234 (2014)
3. S. Patel, L. Kumar, N. Singh, Metformin and epithelial ovarian cancer therapeutics. *Cell. Oncol.* **38**, 365–375 (2015)
4. D. Cvetkovic, Early events in ovarian oncogenesis. *Reprod. Biol. Endocrinol.* **1**, 68 (2003)
5. F. Odicino, S. Pecorelli, L. Zigliani, W. T. Creasman, History of the FIGO cancer staging system. *Int. J. Gynaecol. Obstet.* **101**, 205–210 (2008)
6. E. C. Kohn, L. A. Travers, J. Kassis, U. Broome, J. Klominek, Malignant effusions are sources of fibronectin and other promigratory and proinvasive components. *Diagn. Cytopathol.* **33**, 300–308 (2005)
7. I. Matte, D. Lane, C. Laplante, C. Rancourt, A. Piche, Profiling of cytokines in human epithelial ovarian cancer ascites. *Am. J. Cancer Res.* **2**, 566–580 (2012)
8. M. R. Simpson-Abelson, J. L. Loyall, H. K. Lehman, J. L. Barnas, H. Minderman, K. L. O'Loughlin, P. K. Wallace, T. C. George, P. Peng, R. J. Kelleher Jr., K. Odunsi, R. B. Bankert, Human ovarian tumor ascites fluids rapidly and reversibly inhibit T cell receptor-induced NF-kappaB and NFAT signaling in tumor-associated T cells. *Cancer Immun.* **13**, 14 (2013)
9. I. Matte, D. Lane, D. Bachvarov, C. Rancourt, A. Piche, Role of malignant ascites on human mesothelial cells and their gene expression profiles. *BMC Cancer* **14**, 288 (2014)
10. I. Matte, D. Lane, C. Laplante, P. Garde-Granger, C. Rancourt, A. Piche, Ovarian cancer ascites enhance the migration of patient-derived peritoneal mesothelial cells via cMet pathway through HGF-dependent and -independent mechanisms. *Int. J. Cancer* **137**, 289–298 (2015)
11. H. A. Kenny, C. Y. Chiang, E. A. White, E. M. Schryver, M. Habis, I. L. Romero, A. Ladanyi, C. V. Penicka, J. George, K. Matlin, A. Montag, K. Wroblewski, S. D. Yamada, A. P. Mazar, D. Bowtell, E. Lengyel, Mesothelial cells promote early ovarian cancer metastasis through fibronectin secretion. *J. Clin. Invest.* **124**, 4614–4628 (2014)
12. J. Mikula-Pietrasik, P. Sosinska, M. Kucinska, M. Murias, K. Maksin, A. Malinska, A. Ziolkowska, H. Piotrowska, A. Wozniak, K. Ksiazek, Peritoneal mesothelium promotes the progression of ovarian cancer cells in vitro and in a mice xenograft model in vivo. *Cancer Lett.* **355**, 310–315 (2014)
13. K. Ksiazek, A. Jorres, J. Witowski, Senescence induces a proangiogenic switch in human peritoneal mesothelial cells. *Rejuvenation. Res.* **11**, 681–683 (2008)
14. K. Ksiazek, J. Mikula-Pietrasik, K. Korybalska, G. Dworacki, A. Jorres, J. Witowski, Senescent peritoneal mesothelial cells promote ovarian cancer cell adhesion: the role of oxidative stress-induced fibronectin. *Am. J. Pathol.* **174**, 1230–1240 (2009)
15. J. Mikula-Pietrasik, P. Sosinska, E. Naumowicz, K. Maksin, H. Piotrowska, A. Wozniak, D. Szperek, K. Ksiazek, Senescent peritoneal mesothelium induces a pro-angiogenic phenotype in ovarian cancer cells in vitro and in a mouse xenograft model in vivo. *Clin. Exp. Metastasis* **33**, 15–27 (2016)
16. K. Ksiazek, Mesothelial cell: a multifaceted model of aging. *Ageing Res. Rev.* **12**, 595–604 (2013)
17. J. Mikula-Pietrasik, P. Sosinska, J. Janus, B. Rubis, M. Brewinska-Olchowik, K. Piwocka, K. Ksiazek, Bystander senescence in human peritoneal mesothelium and fibroblasts is related to thrombospondin-1-dependent activation of transforming growth factor-beta1. *Int. J. Biochem. Cell. Biol.* **45**, 2087–2096 (2013)
18. P. Sosinska, J. Mikula-Pietrasik, M. Ryzek, E. Naumowicz, K. Ksiazek, Specificity of cytochemical and fluorescence methods of senescence-associated beta-galactosidase detection for ageing driven by replication and time. *Biogerontology* **15**, 407–413 (2014)
19. J. Mikula-Pietrasik, A. Kuczmarzka, M. Kucinska, M. Murias, M. Wierzchowski, M. Winckiewicz, R. Staniszewski, A. Breborowicz, K. Ksiazek, Resveratrol and its synthetic derivatives exert opposite effects on mesothelial cell-dependent angiogenesis via modulating secretion of VEGF and IL-8/CXCL8. *Angiogenesis* **15**, 361–376 (2012)
20. K. Lessan, D. J. Aguiar, T. Oegema, L. Siebenson, A. P. Skubitz, CD44 and beta1 integrin mediate ovarian carcinoma cell adhesion to peritoneal mesothelial cells. *Am. J. Pathol.* **154**, 1525–1537 (1999)
21. C. Bolitho, M. A. Hahn, R. C. Baxter, D. J. Marsh, The chemokine CXCL1 induces proliferation in epithelial ovarian cancer cells by transactivation of the epidermal growth factor receptor. *Endocr. Relat. Cancer* **17**, 929–940 (2010)
22. K. Fischer, V. Lutz, O. Wilhelm, M. Schmitt, H. Graeff, P. Heiss, T. Nishiguchi, N. Harbeck, H. Kessler, T. Luther, V. Magdolen, U. Reuning, Urokinase induces proliferation of human ovarian cancer cells: characterization of structural elements required for growth factor function. *FEBS Lett.* **438**, 101–105 (1998)
23. N. Said, M. J. Socha, J. J. Olearczyk, A. A. Elmarakby, J. D. Imig, K. Motamed, Normalization of the ovarian cancer microenvironment by SPARC. *Mol. Cancer Res.* **5**, 1015–1030 (2007)
24. Y. Wang, J. Yang, Y. Gao, Y. Du, L. Bao, W. Niu, Z. Yao, Regulatory effect of e2, IL-6 and IL-8 on the growth of epithelial ovarian cancer cells. *Cell. Mol. Immunol.* **2**, 365–372 (2005)
25. Y. Hirashima, H. Kobayashi, M. Suzuki, Y. Tanaka, N. Kanayama, T. Terao, Transforming growth factor-beta1 produced by ovarian cancer cell line HRA stimulates attachment and invasion through an up-regulation of plasminogen activator inhibitor type-1 in human peritoneal mesothelial cells. *J. Biol. Chem.* **278**, 26793–26802 (2003)
26. S. Furukawa, S. Soeda, Y. Kiko, O. Suzuki, Y. Hashimoto, T. Watanabe, H. Nishiyama, K. Tasaki, H. Hojo, M. Abe, K. Fujimori, MCP-1 promotes invasion and adhesion of human ovarian cancer cells. *Anticancer Res.* **33**, 4785–4790 (2013)
27. N. Ahmed, K. L. Stenvers, Getting to know ovarian cancer ascites: opportunities for targeted therapy-based translational research. *Front. Oncol.* **3**, 256 (2013)
28. J. Mikula-Pietrasik, P. Sosinska, K. Maksin, M. G. Kucinska, H. Piotrowska, M. Murias, A. Wozniak, D. Szperek, K. Ksiazek, Colorectal cancer-promoting activity of the senescent peritoneal mesothelium. *Oncotarget* **6**, 29178–29195 (2015)
29. G. D'Andrilli, A. Giordano, A. Bovicelli, Epithelial ovarian cancer: the role of cell cycle genes in the different histotypes. *Open. Clin. Cancer J.* **2**, 7–12 (2008)
30. G. V. Raghuram, P. K. Mishra, Stress induced premature senescence: a new culprit in ovarian tumorigenesis? *Indian J. Med. Res.* **140(Suppl)** S120–S129 (2014)
31. K. Lohani, S. Shetty, P. Sharma, V. Govindarajan, P. Thomas, B. Loggie, Pseudomyxoma peritonei: inflammatory responses in the peritoneal microenvironment. *Ann. Surg. Oncol.* **21**, 1441–1447 (2014)
32. S. Judge, P. Thomas, V. Govindarajan, P. Sharma, B. Loggie, Malignant peritoneal mesothelioma: characterization of the inflammatory response in the tumor microenvironment. *Ann. Surg. Oncol.* **23**, 1496–1500 (2016)
33. C. Fripiat, Q. M. Chen, S. Zdanov, J. P. Magalhaes, J. Remacle, O. Toussaint, Subcytotoxic H2O2 stress triggers a release of transforming growth factor-beta 1, which induces biomarkers of cellular senescence of human diploid fibroblasts. *J. Biol. Chem.* **276**, 2531–2537 (2001)

34. R. Tremain, M. Marko, V. Kinnimulki, H. Ueno, E. Bottinger, A. Glick, Defects in TGF-beta signaling overcome senescence of mouse keratinocytes expressing v-Ha-ras. *Oncogene* **19**, 1698–1709 (2000)
35. G. Yang, D. G. Rosen, Z. Zhang, R. C. Bast Jr., G. B. Mills, J. A. Colacino, I. Mercado-Uribe, J. Liu, The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. *Proc. Natl. Acad. Sci. USA* **103**, 16472–16477 (2006)
36. M. Miyazaki, E. Gohda, K. Kaji, M. Namba, Increased hepatocyte growth factor production by aging human fibroblasts mainly due to autocrine stimulation by interleukin-1. *Biochem. Biophys. Res. Commun.* **246**, 255–260 (1998)
37. M. Mariani, M. McHugh, M. Petrillo, S. Sieber, S. He, M. Andreoli, Z. Wu, P. Fiedler, G. Scambia, S. Shahabi, C. Ferlini, HGF/c-met axis drives cancer aggressiveness in the neo-adjuvant setting of ovarian cancer. *Oncotarget* **5**, 4855–4867 (2014)
38. A. Elkhattouti, M. Hassan, C. R. Gomez, Stromal Fibroblast in Age-Related Cancer: Role in Tumorigenesis and Potential as Novel Therapeutic Target. *Front. Oncol.* **5**, 158 (2015)
39. S. Domcke, R. Sinha, D. A. Levine, C. Sander, N. Schultz, Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat. Commun.* **4**, 2126 (2013)
40. A. R. Davalos, J. P. Coppe, J. Campisi, P. Y. Desprez, Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev.* **29**, 273–283 (2010)