Chlamydia trachomatis induces an upregulation of molecular biomarkers podoplanin, Wilms’ tumour gene 1, osteopontin and inflammatory cytokines in human mesothelial cells

Anna De Filippis,1 Elisabetta Buommino,1 Marina Di Domenico,2 Antonia Feola,2 Raffaella Brunetti-Pierri3 and Antonietta Rizzo1,∗

Abstract

Chlamydia trachomatis is the most prevalent infection of the genital tract in women worldwide. C. trachomatis has a tendency to cause persistent infection and induce a state of chronic inflammation, which has been reported to play a role in carcinogenesis. We report that persistent C. trachomatis infection increases the expression of inflammatory tumour cytokines and upregulates molecular biomarkers such as podoplanin, Wilms’ tumour gene 1 and osteopontin in primary cultures of mesothelial cells (Mes1) and human mesothelioma cells (NCI). Infection experiments showed that Mes1 and NCI supported the growth of C. trachomatis in vitro, and at an m.o.i. of 4, the inclusion-forming units/cell showed many intracellular inclusion bodies after 3 days of infection. However, after 7 days of incubation, increased proliferative and invasive activity was also observed in Mes1 cells, which was more evident after 14 days of incubation. ELISA analysis revealed an increase in vascular endothelial growth factor, IL-6, IL-8, and TNF-α release in Mes1 cells infected for a longer period (14 days). Finally, real-time PCR analysis revealed a strong induction of podoplanin, Wilms’ tumour gene 1 and osteopontin gene expression in infected Mes1 cells. The aim of the present study was to investigate the inflammatory response elicited by C. trachomatis persistent infection and the role played by inflammation in cell proliferation, secretion of proinflammatory cytokines and molecular biomarkers of cancer. The results of this study suggest that increased molecular biomarkers of cancer by persistent inflammation from C. trachomatis infection might support cellular transformation, thus increasing the risk of cancer.

INTRODUCTION

Cancer continues to be the leading cause of death in developed countries. The role of bacteria as mediators of oncogenesis is an intriguing area of research, but how they influence disease development in the human host is poorly elucidated. Specific bacteria species can cause cancer through different and complex mechanisms. For some bacterial species this relationship is well established, whereas for other pathogens it is somewhat weak. An important common characteristic is the time interval between bacterial infection and cancer development [1]. Several bacteria can cause chronic infections or produce toxins that disturb the cell cycle and result in altered cell growth or DNA damage. Deficiencies in DNA-damage signalling and repair pathways lead to genetic instability, which in turn might enhance oncogenesis [2].

Chlamydia trachomatis, an obligate intracellular Gram-negative bacterium, exhibits a biphasic life cycle: metabolically inactive elementary bodies (EBs) infect host cells and differentiate into metabolically active, replicating reticulate bodies (RBs) within a membrane-bound vacuole, which is called inclusion. C. trachomatis has a tendency to cause persistent infections that are usually asymptomatic but can lead to genital infections in women and generate cervicitis, cervical erosion, pelvic inflammatory disease, fallopian tube scarring and infertility [3]. In recent epidemiological studies, Chlamydia infection has been linked to an increased risk of cervical cancer [4]. It has been suggested that cells infected by Chlamydia have a much higher risk of neoplastic transformation because the pathogen is able to cause persistent inflammation and tissue damage and inhibit apoptosis in
infected cells [5]. C. trachomatis modulates host cell functions in ways that convey benefits to the pathogen but have severe consequences for the fate of the host cells. This may be of particular importance during chronic infection [6]. C. trachomatis triggers the activation of oncogenic Ras-Raf-MEK-ERK pathway components [7] and the production of reactive oxygen species to support its growth [8]. Bacterial pathogens such as Mycobacterium tuberculosis, Shigella flexneri, Listeria monocytogenes and Helicobacter pylori are known to modify histones and remodel the chromatin architecture of host cells during infections, thus manipulating host transcriptomes, e.g. immune responses [9]. Furthermore, Chlamydia pneumoniae induces modifications in histones H3 and H4, which play an important role in cytokine production [10]. C. trachomatis infecting epithelial cells is the most prevalent infection of the genital tract in women worldwide [11]. Epithelial cells of the human genital tract can secrete different proinflammatory cytokines and chemokines in response to abnormal conditions, such as infections [12].

Podoplanin (PL) is a type-I transmembrane sialomucin-like glycoprotein that is expressed in the tumour cells of various types of cancer, such as vascular tumour, malignant mesothelioma, central nervous system tumour and squamous cell carcinoma [13]. PL was recently shown to be over-expressed in adenocarcinoma of the lung and in breast cancer, where it was associated with tumour progression, metastasis and a poor prognosis [14, 15]. Osteopontin (OPN) is a phosphoglycoprotein that binds the integrin and CD44 families of receptors [16]; it is a secreted plasma marker with an established role in tumour transformation, invasion and progression [17–19]. OPN has been considered one of the most promising biomarkers for various cancers [20]. The Wilms’ tumour gene 1 (WT1) is an important regulator of cell growth and development in the embryo kidney, adult urogenital system and central nervous system [21]; however, the transcription factor WT1 has been found to be activated in many human neoplasias, including mesothelioma cells [22, 23]. WT1 expression has been reported to be markedly low in cells of normal healthy individuals, with the exception of the CD34+ haematopoietic progenitors [24].

The use of molecular biomarkers for the early diagnosis of cancer has been a long-standing objective. Given the association between infection, inflammation and cancer, and since C. trachomatis is able to cause persistent inflammation and has been associated with an increased risk of cervical cancer, the inflammatory response elicited by human epithelial cells during C. trachomatis infection and the effects of this inflammatory response on cell proliferation and carcinogenesis need to be elucidated.

In the present study, we explored the hypothesis that C. trachomatis persistent infection enhances cell proliferation and secretion of cytokines that might elicit molecular biomarkers of cancer involved in carcinogenesis.

METHODS

Cell culture and treatments

HeLa cells, a human cervical adenocarcinoma epithelial cell line (ATCC CCL2; Rockville, MD), were propagated under standard culture conditions. Cell cultures were incubated with Dulbecco’s modified Eagle’s medium (DMEM; Euroclone), supplemented with 10 % foetal bovine serum (FBS; Gibco BRL, Milan, Italy), 2 mM L-glutamine, 0.1 mg ml⁻¹ penicillin, and 0.1 mg ml⁻¹ streptomycin at 37 °C in a humidified atmosphere of 5 % CO₂.

NCI-H2452 (NCI) human mesothelioma cells (ATCC CRL-5946; Manassas), an established cell line, were maintained in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10 % FBS, 0.1 mg ml⁻¹ penicillin, 0.1 mg ml⁻¹ streptomycin and 2 mM L-glutamine at 37 °C, 5 % CO₂. NCI cells were used as a positive control for human mesothelioma. Cell viability was assessed by means of the Trypan blue dye exclusion method (Sigma-Aldrich, Milan, Italy).

Primary cultures of mesothelial cells (Mes1) were isolated and developed from a pleural biopsy of a patient who was cytologically, histologically and immunohistochemically confirmed as having non-malignant pleural mesothelioma, as previously reported [25]. In brief, Mes1 and NCI cells were analysed using reverse transcriptase-PCR (RT-PCR) to determine the expression of carcinoembryonic antigen (negative marker), WT1, mesothelin and calretinin. Mesothelin and calretinin are proteins that are strongly expressed in mesothelial cells and mesotheliomas, whereas WT1 is expressed mainly in mesothelioma cells [26]. The expression of mesothelin and calretinin in our primary cell culture confirmed mesothelial differentiation; WT1 was strongly expressed in NCI cells (data not shown). Mes1 cells were incubated in growth medium comprising a 1:1 composition of DMEM and Ham’s F12 medium (Gibco Invitrogen, Milan, Italy) supplemented with 20 % FCS, 0.1 mg ml⁻¹ penicillin, 0.1 mg ml⁻¹ streptomycin, 10 μg ml⁻¹ epidermal growth factor, 5 mg ml⁻¹ insulin and 0.2 mg ml⁻¹ hydrocortisone. Cell cultures were incubated at 37 °C in a humidified atmosphere of 5 % CO₂ for 14 days to achieve 75 % confluence.

Propagation and titration of C. trachomatis

C. trachomatis serovar D (UW-3; ATCC VR-885) was propagated in HeLa cell monolayers as previously reported [27] and quantified as inclusion forming units (IFUs) as described previously [28]. In brief, C. trachomatis was inoculated onto a pre-formed monolayer of HeLa cells, centrifuged at 1000 g for 60 min at 25 °C and incubated at 37 °C with 5 % CO₂ for 1 h. Supernatants were replaced with a growth medium consisting of DMEM containing 1 μg ml⁻¹ cycloheximide. Infected cultures were incubated for 48 h at 37 °C in 5 % CO₂. C. trachomatis was harvested by disrupting HeLa cells with glass beads followed by sonicatation and centrifugation at 250 g to remove cellular debris. Supernatants containing C. trachomatis were further centrifuged at 20 000 g for 20 min to pellet EBs. The EB pellet was then suspended in sucrose-phosphate-glutamate buffer, aliquoted and stored at −70 °C.
Infectivity titres of chlamydial stocks were evaluated by titration of the IFUs per millilitre in HeLa cells. These titres were used to determine the infective doses for the cell line studied. Cell cultures and chlamydial stocks were confirmed to be free of Mycoplasma infections by using 4,6-diamidino-2-phenylindole fluorescent staining (Sigma-Aldrich, Milan, Italy). Contamination with Mycoplasma was also excluded by Mycoplasma-PCR using specific primers (MWG-Biotech, Martinsried, Germany).

Infection protocol
Mes1 and NCI cells were seeded onto the coverslips in 24-well plates at a density of $5 \times 10^4$ cells per well. The medium was refreshed after 2 days and cells were grown in the growth medium without antibiotics at 37°C in 5% CO2. The cells were then infected with C. trachomatis at an m.o.i. of 4 IFU per cell or mock-infected (medium alone). One m.o.i. is equivalent to one infectious EB per HeLa cell. The plates were centrifuged at 1000 g for 60 min and incubated at 37°C with 5% CO2 in air for 24 h. For persistent C. trachomatis infection, 24 h post infection, 250 ng ml$^{-1}$ doxycycline was added to both uninfected and infected cells, and cells were allowed to grow for 3, 7 and 14 days. (A preliminary study showed that this m.o.i. was optimal for the cell lines analysed and for all times studied, with no toxic effect on the culture medium.)

Morphological analysis
To determine whether Mes1 and NCI cells were capable of supporting the growth of C. trachomatis in vitro, and to determine the level of infection at 3, 7 and 14 days, the infected cells were fixed with 100% methanol and stained for the inclusion bodies using an FITC-conjugated anti-major outer membrane protein (MOMP) of C. trachomatis (MicroTrak, Trinity Biotech, NY). The morphological features of Mes1 and NCI cells infected with C. trachomatis were examined at a magnification of ×400 by confocal fluorescence microscopy (AxiOSkop 2 ZEISS; Carl Zeiss, Milan, Italy). Each culture was carried out in triplicate.

Cell viability
Cell viability was determined using methyltetrazolium (MTT). Mes1 and NCI cultures were incubated with DMEM alone (negative control) or with C. trachomatis (m.o.i. of 4) at 37°C in 5% CO2. The number of living cells was determined after 3, 7 and 14 days by colorimetric MTT assay [3-(4,5-dimethyl-2,5-thiazolyl)-2-5 diphenyltetrazolium bromide; Sigma-Alrlich] according to the procedure of Boonyanugomol et al. [29]. The absorbance of a formazan product in the tissue culture media was measured at 650 nm using a microplate reader. MTT assay data of the cells infected with C. trachomatis and those of the controls were confirmed by counting infected and uninfected cells in a Bürker chamber. For viability evaluated by microscopy examination, the cells were observed at a magnification of ×200 (CK40 Olympus Microscope). Each culture was done in triplicate and each test carried out in triplicate. The data are expressed as the means±SD.

Cell invasion assay
Cell invasion assays were carried out in Boyden chambers under serum-free conditions as previously described [30]. The 10 µm pore-size polycarbonate filters were coated with 5 µg ml$^{-1}$ fibronectin and then with 25 µg ml$^{-1}$ Matrigel (BD Biosciences). After 3, 7 and 14 days of C. trachomatis incubation, Mes1 and NCI cells were trypsinized and placed in the upper compartment of the Boyden chamber in serum-free medium, and FBS 10% was placed in the lower compartment as the chemoattractant. Cells were allowed to attach and spread at 37°C in 5% CO2 for 24 h. After incubation, the cells on the upper surface of the filter were removed completely by wiping with a cotton swab, while those that had passed through the Matrigel to the lower surface of the filter were fixed in ethanol, stained with haematoxylin and counted in 10 random fields per filter at ×200. In parallel, the control cells were assessed for viability and counted with Trypan blue. The results were expressed as percentages of invasion. The number of cells that had invaded was normalized to analyse the effects on uninfected cell viability. Each culture was done in triplicate and each test carried out in triplicate.

Measurement of cytokine concentrations
To investigate whether C. trachomatis (m.o.i. of 4) induced vascular endothelial growth factor (VEGF), IL-6, IL-8, and TNF-α production, infected Mes1 and NCI cells were cultured in six-well plates (Falcon; Becton Dickinson Labware; Franklin Lakes, NJ) at a concentration of 104 cells ml$^{-1}$ and incubated in 5% CO2 at 37°C. Each culture was carried out in triplicate. As a positive control, we infected the NCI cells with C. trachomatis. After 3, 7 and 14 days of incubation in 5% CO2 at 37°C, the cultures were centrifuged at 450 g at 4°C for 10 min and the culture media were collected and analysed for VEGF, IL-6, IL-8 and TNF-α by ELISA, according to the manufacturer’s recommended procedure (R and D Systems, Minneapolis, MN). The ODs of the wells were determined using a microplate reader (Eppendorf BioPhotometer) set at 450 nm with wavelength correction set at 540 nm. The cytokine concentrations were determined in triplicate in each culture. The data are expressed as means±SD, after the exclusion of outliers.

RNA isolation
Semi-confluent Mes1 (1$\times 10^6$ per well) and NCI (4$\times 10^5$ per well) cells were infected with C. trachomatis for 3, 7 and 14 days. Total RNA was extracted from Mes1 and NCI cells, infected or non-infected with C. trachomatis, using the High Pure RNA Isolation Kit (Roche Diagnostics, Milan, Italy). Total RNA was quantified by measuring the OD with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE).

Reverse transcriptase PCR
Three hundred nanograms of total cellular RNA were reverse-transcribed (Expand Reverse Transcriptase, Roche Diagnostics, Indianapolis, IN) into complementary DNA (cDNA)
using random hexamer primers (Roche Diagnostics) at 42°C for 45 min according to the manufacturer’s instructions.

**Real-time quantitative PCR**

Real-time quantitative PCRs for OPN, WT1 and PPL were carried out with the LC FastStart DNA Master SYBR Green kit using 2 µl cDNA, corresponding to 10 ng total RNA in a 20 µl final volume, 3 mM MgCl$_2$ and 0.5 µM sense and anti-sense primers (Table 1). The cycling conditions were as follows: one cycle of denaturation at 95°C for 3 min, 40 cycles at 94°C for 5 s and 53°C for 11 s for OPN [31]; one cycle of denaturation at 95°C for 3 min, 40 cycles at 95°C for 5 s and 56°C for 8 s for WT1 [32]; one cycle of denaturation at 95°C for 3 min, 33 cycles at 94°C for 15 s and 56°C for 20 s for PPL [33]. After amplification, melting curve analysis was performed by heating to 95°C for 15 s with a temperature transition rate of 20°C/s, cooling to 60°C for 15 s with a temperature transition rate of 20°C/s, and then heating the sample at 0.1°C/s to 95°C. The results were analysed using LightCycler software (Roche Diagnostics). The fragments were separated on 1.5% agarose gel. The standard curves for each primer pair were established with serial dilutions of cDNA. Quantification was based on the threshold cycle values, measured in the early stage of the exponential phase of the reaction. All reactions were run in triplicate.

**Statistical analysis**

All experiments were performed three times and the measurements taken in triplicate, unless otherwise indicated. The results are expressed as means±s.d. Outliers were excluded from the analysis where appropriate. Student’s t-test was used to determine statistical differences between the means and $P<0.05$ was considered a significant difference.

**RESULTS**

**Susceptibility of Mes1 and NCI cells to C. trachomatis infection**

Infection experiments showed that Mes1 and NCI cells supported the growth of C. trachomatis in vitro. The cells infected with C. trachomatis were determined by confocal fluorescence microscopy using an anti-chlamydia monoclonal antibody. Determinations were performed with an m.o.i. of 4 at 3, 7 and 14 days post infection with infection rates from 50% to 70% measured using HeLa cells. No significant cell lysis was observed in infected Mes1 and NCI cells. Fig. 1 shows infected cells with typical inclusion bodies. Both Mes1- (Fig. 1a–d) and NCI- (Fig. 1e–h) infected cells showed many intracellular inclusion bodies 3 days after infection while after 7 days of incubation an increase in intracellular inclusion bodies was observed, which was more evident after 14 days of incubation. Moreover, C. trachomatis was able to complete its biphasic developmental cycle, as indicated by the ability of supernatants from infected Mes1 and NCI cells to re-infect highly susceptible epithelial cell lines such as HeLa cells (data not shown).

The effect of C. trachomatis infection on Mes1 and NCI cell growth evaluated by MTT assay is shown in Fig. 1(i). Cells were infected and analysed at 3, 7 and 14 days. After the shortest period of incubation (3 days), compared to uninfected control cells, a greater increase in infected Mes1 cell viability was observed (114.2%); while at 7 and 14 days the infected Mes1 cell viabilities were 72.1 and 95.3%, respectively. After 3, 7 and 14 days, a slight increase in infected NCI cell viability was observed (8.8%, 7.5 and 6.7%, respectively) compared to uninfected control cells. These results were confirmed by cell counting (data not shown).

**C. trachomatis infection increases cell invasion**

To support the results obtained, we investigated the ability of C. trachomatis to increase Mes1 and NCI cell invasion. Large numbers of cells were found on the lower surface of the membrane, thus showing the strongly invasive properties of Mes1 and NCI cells through a Matrigel-coated filter. Mes1 cells infected with C. trachomatis after only 3 days of incubation under the polycarbonate membrane already showed cell invasion (26%). After 7 days, Mes1 cells showed significantly greater cell invasion (53%), while after 14 days the invasion percentage of Mes1 cells was found to increase significantly in cells infected with C. trachomatis (87%) (Fig. 2). In addition, NCI cells used as a positive control for human mesothelioma already showed cell invasion after 3 days of incubation both in infected and uninfected cells; at 7 and 14 days no further significant changes were seen. This shows a time-dependent effect of infection with C. trachomatis on the migration of Mes1 cells through Matrigel.

**C. trachomatis infection increases the expression of proinflammatory cytokines in Mes1 and NCI cells**

To measure the release of proinflammatory cytokines, Mes1 and NCI cells were grown in 24-well plates and infected or

---

**Table 1. Human sense and antisense primer sequences and expected PCR products (bp)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense and antisense sequences</th>
<th>Expected product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPN</td>
<td>5’-CACCTGTGCCATACCGTTAAAC-3’</td>
<td>532</td>
</tr>
<tr>
<td></td>
<td>5’-GGTTAGTGCTCTGCTGTACATC-3’</td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>5’-CTCTTGTACGGTCGCCATCT-3’</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>5’-CAGCCTGAGTTTGGTCATG-3’</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>5’-CACCTCAAGCAACCTCAAC-3’</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>5’-ACAGGCCAAGTGTGGAAGC-3’</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>5’-TGACGGGTTGACCACACTGTGCCCATCTA-3’</td>
<td>661</td>
</tr>
<tr>
<td></td>
<td>5’-CTGAAGACATTTGCGGACGATGGAGG-3’</td>
<td></td>
</tr>
</tbody>
</table>
not with C. trachomatis (m.o.i. of 4). The levels of VEGF, IL-6, IL-8, and TNF-α proteins secreted by Mes1 and NCI cells were measured at 3, 7 and 14 days (Fig. 3). After 3 days of incubation, C. trachomatis-infected Mes1 cells already showed an increase in cytokine levels. However, a stronger release was observed after a longer period of incubation (7 and 14 days). In particular, C. trachomatis infection induced a significant protein release after 7 days, as follows: VEGF, IL-6, IL-8, and TNF-α secretions were 1856±232, 1107±138.3, 1298±162.2 and 6.2±0.78 ng ml⁻¹, respectively, versus negative controls 341±42.6, 280±35, 296±37 and 1.9±0.24 ng ml⁻¹, respectively (Fig. 3a–d). Similarly, in Mes1 cells infected with C. trachomatis for 14 days, a strong increase in VEGF, IL-6, IL-8 and TNF-α secretion was observed (2452±306.5, 1210±151.2, 1309±240.4 and 7.7±0.96 pg ml⁻¹, respectively) versus negative controls (402±50.2, 395±49.3, 351±43.8 and 2.2±0.8 pg ml⁻¹, respectively) (Fig. 3a–d).

C. trachomatis infection induces PL, OPN and WT1 gene expression

To investigate whether chlamydial infection might promote cellular transformation we analysed the PL, OPN and WT1 gene expressions, all known markers involved in tumour progression. Fig. 4(a) clearly demonstrates that 7 and 14 days after infection with C. trachomatis, Mes1 cells showed a strong increase in the PL gene expression (140 and 116 %, respectively) compared to uninfected Mes1. Also the OPN gene expression had strongly increased in C. trachomatis-infected Mes1 cells, as shown in Fig. 4(b), and the greatest increase was reached 14 days after infection (161 %) compared to uninfected Mes1. As shown in Fig. 4(c), the WT1 gene expression had already increased by 125 % after 7 days compared to uninfected cells. A longer period of infection (21 days) was not investigated since Mes1 cell morphology had modified and showed typical features of cell suffering (data not shown). Finally, when the PL, OPN and WT1 gene expressions were analysed in C. trachomatis-infected NCI cells, the results obtained were similar to NCI cells (positive control) (Fig. 4a–c). Quantitative PCR analyses of OPN, PL and WT1 showed that the underlying RNA templates were highly amplified, as indicated by the curve analysis (Fig. 5).

DISCUSSION

C. trachomatis causes permanent cytological changes in cultured cells because of its ability to reside and replicate within the host cell. In our study, Mes1 cells infected with C. trachomatis showed many intracellular inclusion bodies, confirming that the micro-organism was able to invade and replicate in this cell type. Chlamydial infection can induce hyperproliferation due to extensive tissue remodelling during and after the destructive events associated with infection. In accordance with different reports showing the ability of C. trachomatis infection to increase cell proliferation [34], we demonstrated an increased proliferative activity in C. trachomatis-infected Mes1 cells. Interestingly, it has been found that cells infected with C. trachomatis are resistant to apoptosis [35], a critical cellular mechanism that protects cells from tumour transformation. Chlamydia infection promotes host DNA damage and proliferation but impairs the DNA-damage response. The orchestrated deregulation of host cell signalling and perturbations to host cell chromatin lead to the enforced survival of damaged host cells, which is likely to predispose them to transformation [6].
**Fig. 2.** Effect of *C. trachomatis* on Mes1 and NCI cells invasion. Mes1 and NCI cells infected or not with *C. trachomatis* (Ct; m.o.i. of 4) were plated onto a Matrigel modified Boyden chamber. Cells were allowed to attach and spread for 24 h. Only cells that had passed through the Matrigel were stained and counted. The average number of cells per field is expressed as a percentage of the control after normalizing for cell number. The results are the means±SD of three replicates in three independent experiments. The asterisks indicate a statistically significant difference between *C. trachomatis*-infected Mes1 cells versus uninfected cells; **P<0.01.

**Fig. 3.** Effect of *C. trachomatis* on VEGF (a), IL-6 (b), IL-8 (c) and TNF-α (d) secretion in Mes1 and NCI cells infected or not with *C. trachomatis* (Ct; m.o.i. of 4) after 3, 7 and 14 days. Data are means±SD of three replicates in three independent experiments. The asterisks indicate a statistically significant difference between *C. trachomatis*-infected cells versus uninfected cells; *P<0.05, **P<0.01.
Chlamydia infections induce a state of persistent inflammation, which increases the risk of tumour development [36, 37]. It has also been reported that *C. trachomatis* infection increases the risk of cervical cancer [38]. Our study showed that infection with *C. trachomatis* induced a strong inflammatory response in epithelial prostate cells that leads to pro-angiogenic (VEGF) and pro-metastatic cytokine overexpression, such as IL-6, IL-8 and TNF-α. VEGF is a key signalling molecule in the induction of vessel growth, a process termed angiogenesis. Angiogenesis is an essential process involved in the development and progression of prostate cancer [39]. Importantly, the expression of VEGF, IL-6 and IL-8 has been shown to correlate directly with the malignant potential of prostate cancer [40]. IL-6 is a multifunctional inflammatory cytokine that regulates inflammation and can create a cellular microenvironment that is beneficial for cancer growth [41]. IL-8 is a proinflammatory CXC chemokine, and its production is correlated with the metastatic potential of tumour cells [42]. Our results are also in accordance with those of Drott *et al.* [43, 44], who found that prostate epithelial cells can produce IL-8 and IL-6 in response to *Propionibacterium* and *Mycoplasma* infections. TNF-α is a mediator of acute and chronic inflammation, which has been detected in breast, prostate and ovarian carcinomas. In prostate cancer cells, TNF-α appears to be involved in the autocrine and paracrine regulation of cell growth [45]. Our results show that the expression of TNF-α in Mes1 cells increases significantly during *C. trachomatis* infection.

Blood tests as molecular biomarkers for an early detection and monitoring of progression are an objective in cancer diagnostics. It has been suggested that PL may act as a mediator of tumour cell invasion and metastasis, although its biological function is not yet clearly understood [46]. The presence of this protein in tumour cells, which seems to be expressed in aggressive tumours with a greater invasive and metastatic potential, is useful for a pathological diagnosis [47]. PL expression is somewhat higher in early-stage cancer. It is believed that PL in fibroblasts acts as a tumour
results, we analysed another marker of tumour progression, WT1, and demonstrated a significant upregulation of the WT1 gene expression in infected Mes1 cells. WT1 has been implicated in the regulation of cell survival, proliferation and differentiation and may function both as a tumour suppressor and as an oncogene [55]. There is increasing evidence linking the over-expression of the WT1 gene to tumour [56, 57]. As chronic chlamydial infections are characterized by persistence, understanding the long-term effects of bacterial infection has become increasingly important as a possible means of cellular transformation prevention. Because cancer usually has a poor prognosis, there is an urgent need to develop early diagnostic markers and effective therapies against chronic C. trachomatis infections. Although many questions remain, further information on the role of C. trachomatis in cellular transformation could be provided by studies using additional markers of infection and inflammation.

To our knowledge, this is the first report of C. trachomatis infection in human mesothelial cells supporting the idea that persistent inflammation by C. trachomatis infection increases molecular biomarkers of cancer facilitated by inflammation.

Funding information
The authors received no specific grant from any funding agency.

Acknowledgements
This study was supported by Department of Experimental Medicine, Section of Microbiology and Clinical Microbiology, Faculty of Medicine and Surgery – Second University of Naples.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References


Edited by: R. Manganelli and S. V. Gordon

---

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.