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Tumor-associated macrophages promote invasion *via* Toll-like receptors signaling in patients with ovarian cancer

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ABSTRACT

Tumor-associated macrophages (TAMs) derived from peripheral blood monocytes recruit into tumor microenvironment and display functions associated with tumor progression. The mechanisms by which TAMs display roles that associated with the invasion ability of ovarian cancer have not been well investigated. In our research, we found abundant TAMs infiltrate in ovarian cancer compared with benign ovarian tumor tissues. Levels of matrix metalloproteinase (MMP)-2, MMP-9 and MMP-10, and Toll-like receptors (TLRs) signaling proteins were evaluated in ovarian cancer. The high level of TAMs was associated with metastasis and advance of patients with ovarian cancer. TAMs and ovarian cancer cell line SKOV3 were cocultured *in vitro*, MMPs level and the invasion ability of SKOV3 cells were significantly up-regulated. The coculture process was correlated with the activation of TLRs signaling and downstream nuclear factor (NF)-κB p65 and microtubule-associated proteins (MAPs) kinases pathway in SKOV3. In addition, pre-incubation with TLRs signaling inhibitors remarkably suppressed invasion ability of SKOV3. Levels of TLRs signaling pathways proteins were also down-regulated in this blocking process. These findings demonstrated that TAMs promoted up-regulation of MMP-2, MMP-9 and MMP-10 expressions and enhanced ovarian cancer cells invasion *via* TLRs signaling pathway. We conclude that TAMs could enhance ovarian cancer cells invasion and ultimately promote ovarian cancer progression.

1. Introduction

Epithelial ovarian cancer (EOC) is a highly heterogeneous disease and remains the most lethal gynaecological malignancy around the world [1]. A noteworthy feature of advanced EOC is the occurrence of widespread peritoneal metastases in initial diagnosis. Recent researches declared that the metastasis and invasion of EOC play a vital role for its poor prognosis [2]. As invasion process is notably for tumor progression, the function of matrix metalloproteinases (MMPs) family is crucial during chronic inflammation and remodeling of damaged tissues. In particular, MMP-2, MMP-9 and MMP-10 are family members of zinc-dependent *endo*-peptidases and regarded as crucial enzymes for cell progression, invasion and metastasis due to its capability to degrade components of the extracellular matrix during physiological and pathological processes [3,4].

Compelling researches have occurred in recent years that the tumor microenvironment showed an important role in tumor progression [5,6]. Circulating monocytes can be recruited and differentiated into M1 or M2-polarized macrophages as a result of activation by some molecular factors in tumor microenvironment. Macrophages are heterogeneous cell population that responds to diverse microenvironmental signals. M1-polarized macrophages are potent effector cells that kill tumor cells and microorganisms. In contrast, M2-polarized macrophages have the interleukin (IL)-12^{low}, IL-23^{low} and IL-10^{high} phenotype and highly expressed many receptors, such as scavenger receptor (SR, CD68) and mannose receptor (MR, CD206) [7,8]. Recruited monocytes were shown to mostly be differentiated to tumor-associated macrophages (TAMs), which have the polarized M2 phenotype. TAMs receive signals within the tumor microenvironment and release various factors

Abbreviations: DAMPs, danger-associated molecular patterns; EOC, epithelial ovarian cancer; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FSL-1, fibroblast-stimulating lipopeptide-1; HKLM, heat-killed *Listeria monocytogenes*; IL, interleukin; IRAK, IL-1R-associated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAPs, microtubule-associated proteins; MMPs, matrix metalloproteinases; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor -kappa B; PAMPs, pathogen-associated molecular patterns; PBS, phosphate-buffered saline; PDTC, pyrrolidine dithiocarbamate; PMA, phor-bol-12-myristate-13-acetate; TAMs, tumor-associated macrophages; TANK, TRAF family member-associated NF-κB activator; TLRs, Toll-like receptors; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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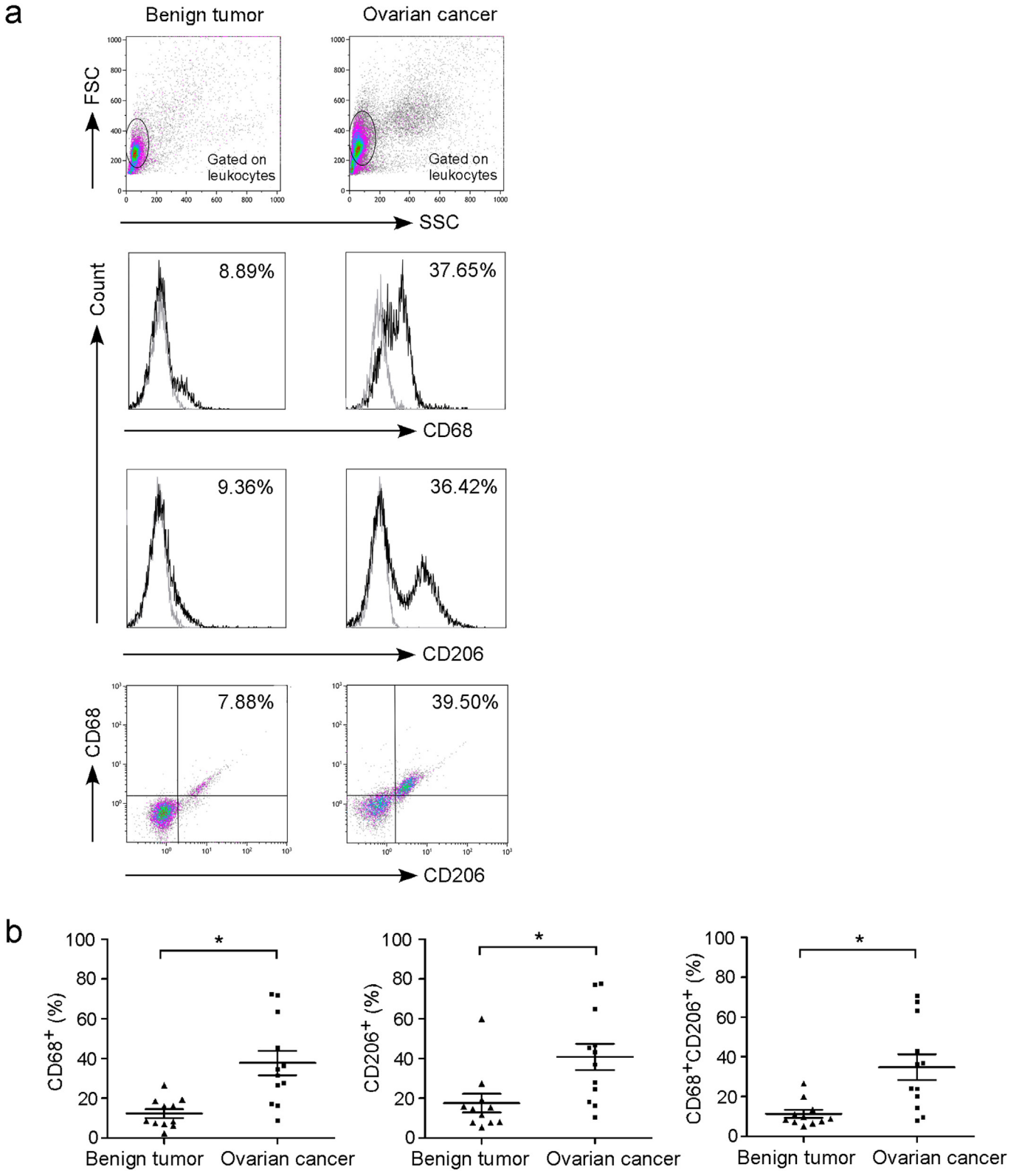

Fig. 1. High percentage of TAMs in patients with ovarian cancer. (a) Flow charts of $CD68^+$ cells, $CD206^+$ cells and $CD68^+CD206^+$ cells from benign tumor controls and ovarian cancer patients. There were higher percentages of $CD68^+$ cells, $CD206^+$ cells and $CD68^+CD206^+$ cells in ovarian cancer than benign ovarian tumor tissues. (b) The percentages of $CD68^+$ cells, $CD206^+$ cells and $CD68^+CD206^+$ cells in ovarian cancer ($n = 12$) and benign ovarian tumor tissues ($n = 11$) by flow cytometry. $^*p < 0.05$ *vs.* benign ovarian tumor.

to promote angiogenesis, suppress antitumor immunity and facilitate tumor cells invasion [9–11]. Previous observations have showed that TAMs played an important role in regulating cancer progression related factors [12–14]. However, the exact mechanisms by which TAMs cells influence invasion ability of ovarian cancer are not well investigated.

Toll-like Receptors (TLRs) are important in innate immunity and expressed in tumor cells in addition to immunocytes. They recognize