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Co-culture of ovarian cancer stem-like cells with macrophages induced SKOV3 cells stemness via IL-8/STAT3 signaling

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ABSTRACT

Among recent concepts in the cancer biology field, the tumor microenvironment is highly associated with cancer stem cells, and plays a key role in tumor progression. This study aimed to explore the mechanism that the stemness induction of SKOV3 cell line by macrophages derived from THP-1 cells, which was co-cultured with SKOV3-derived ovarian cancer stem-like cells (OCSLCs). Sphere formation, soft agar colony formation, and expression levels of CD133 and CD44 were assessed to reflect OCSLC properties. ELISA was used to evaluate secretion profile changes in macrophages co-cultured with or without SKOV3-derived OCSLCs. For mechanistic evaluation, rhIL-8, IL-8 neutralizing antibody (IL-8 Ab), signal transducer and activator of transcription 3 (STAT3) shRNA and STAT3 cDNA were used. The results showed that IL-10, VEGF, MMP-9, IL-8 secretion and CD163 and STAT3 expression levels in macrophages co-cultured with OCSLCs were increased compared with those from THP-1 cells, while IL-12 and NO amounts were significantly reduced, reflecting M2 macrophage polarization. Addition of rhIL-8 to THP-1 cell conditioned media promoted M2 macrophage polarization and stemness in SKOV3 cells, which were suppressed by IL-8 Ab addition to co-culture conditioned media. Consistently, overexpression of STAT3 induced M2 macrophage polarization and stemness in SKOV3 cells, which were inhibited by STAT3 knockdown in macrophages from THP-1 cells. Importantly, STAT3 overexpression rescued the effects of IL-8 Ab on M2 macrophage polarization and stemness in SKOV3 cells. These results suggested that stemness induction in SKOV3 cells by macrophages co-cultured with SKOV3-derived OCSLCs involved IL-8/STAT3 signaling.

1. Introduction

Ovarian cancer is the deadliest and most common of all gynecological malignancies, with high mortality [1]. Its prognosis is poor, with an overall 5-year survival rate of ≪40% due to highly metastatic nature. Each year, nearly 230,000 women are diagnosed with ovarian cancer, with more than 150,000 patients succumbing to this malignancy globally [2]. Although treatment with combined debulking surgery and chemotherapy can achieve complete cyto-reduction, unpredictable early relapses are still encountered. Ovarian cancer is therefore an important public health concern.

Cancer stem cells (CSCs) are considered important players in tumor initiation, development, resistance, and recurrence [3]. Our previous study demonstrated that sphere-forming cells (SFCs) of the SKOV3 cell line possess the properties of ovarian cancer stem cells (OCSCs) [4–6]. It was reported that a specific tumor microenvironment (TME) could enable the maintenance and expansion of CSCs [7]. TME is composed by extracellular matrix (ECM) proteins, cytokines, chemokines, and a variety of cell types such as fibroblasts, endothelial cells, and immune cells, which include macrophages as key components [8]. Macrophages show two different polarization types, including the pro-inflammatory classical (M1) and suppressive alternatively activated (M2) types,

Abbreviations: CSC, cancer stem cell; SFC, sphere-forming cell; OCSC, ovarian cancer stem cell; OCSLC, ovarian cancer stem-like cell; TME, tumor microenvironment; ECM, extracellular matrix; TAM, tumor-associated macrophage; CM, conditional medium; OCSLC-CM, conditional medium of ovarian cancer stem-like cell; THP-1-CM, conditional medium of THP-1 macrophages; Co-CM, conditional medium of SKOV3-derived OCSLC/THP-1 macrophage co-cultures

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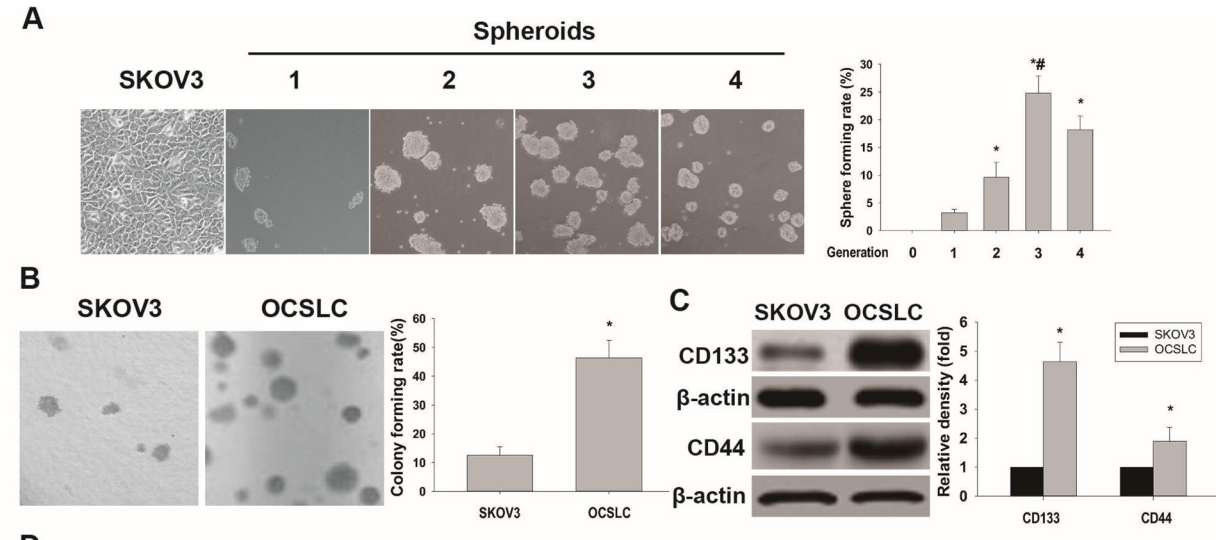

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Cell type	Cell inoculated(n)	Tumor incidence(n/4)	Latency(d)	Tumor volume(mm^3)	Tumor weight(mg)
SKOV3	10^4	0/4			
	10^5	0/4			
	10^6	2/4	15～21	4664±237	792±390
OCSLC	10^3	2/4	15～26	1957±253	524±86
	10^4	4/4	15～17	3358±296*	725±60*
	10^5	4/4	6～9	5444±674*	1230±464*

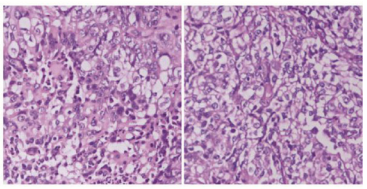

Fig. 1. Comparison of SKOV3 cells and OCSLCs with CSCs characteristics.
A, Representative images of SKOV3 cells (×20, left) and different passages of SKOV3-derived spheroids (×40); quantitation of sphere formation (right). Number 1,2,3,4 represents that the first, second third and fourth generation of spheroids B, Representative images of soft agar colony formation (×10, left); efficiency of colony formation (right). C, Western blot was performed for CD133 and CD44 expression levels, with β-actin as a loading control. D, Incidence, latency, tumor volume, and tumor weight of xenografts in the nude mouse model (left) and representative images of H&E staining (×10, right). $^{*}P \ll 0.05$ *vs* 10^3 SKOV3-derived OCSLC group; $^{\#}P \ll 0.05$ *vs* 10^6 SKOV3 cell group.

depending on the local microenvironment [8]. Previous findings showed that liver cancer stem-like cells induce M2 polarization in tumor-associated macrophages(TAM) [9]. Rao G reported that the interactions between TAM and CD44-positive cancer cells can promote tumorigenicity in colorectal cancer [10], and Yang Ye et al. found that TAM enhanced the invasion of glioma stem-like cells [11], which suggested that TAM may play an important role in the induction of cancer stem cell properties.

Recent studies have demonstrated that a number of signaling pathways directly affect the interactions between TME and CSCs [12–14]. Notably, IL-8, a CXC inflammatory chemokine, is highly involved as a chemoattractant and activator for neutrophils in the immune response [15]. Tumor cells, endothelial cells, infiltrating neutrophils and TAM show significantly increased expression of IL-8 and/or its receptors [16–18], indicating that IL-8 may constitute an important regulatory factor in TME. Meanwhile, IL-8 is known to promote cancer cell proliferation, survival, and migration [19]. A study by Schinke et al. revealed that the IL8 receptor CXCR2 is an adverse prognostic factor in MDS/AML, and a potential therapeutic target against immature leukemic stem cell-enriched cell fractions in acute myeloid leukemia and myelodysplastic syndromes [20]. As a key component of TME, IL-8 produced by macrophages promotes migration and invasion of breast cancer cells [21].

Signal transducer and activator of transcription 3 (STAT3) is involved in proliferation, survival, apoptosis, angiogenesis and metastasis in malignant tumors, as an important oncogene transcription factor [22–25]. Upon stimulation by cytokines, *e.g.* interleukin-6 (IL-6) and IL-8, and growth factors (EGF, HGF, PDGF, *etc.*), STAT3 is phosphorylated at tyrosine 705. STAT3 phosphorylation promotes its homo- and heterodimerization, and the resulting dimer then enters the nucleus where it regulates transcription, leading to increased transcription levels of downstream genes such as Vegf, Bcl-2, BcL-xL, survivin, XIAP, and MMPs [26,27]. In ovarian cancer, p-STAT3 ^{Tyr705} expression is strongly associated with patient prognosis [28]. It was recently demonstrated that IL-8 knockdown reduces STAT3 phosphorylation and nuclear translocation, indicating that IL-8 may trigger p-STAT3 ^{Tyr705} protein expression and induce STAT3 signaling [28]. Our previous study demonstrated that tumor-associated fibroblasts induce stem-like characteristics in SMMC-7721 cells by activating STAT3, indicating that STAT3 signaling may represent a target for therapeutic intervention in TME and CSCs [29]. Based on these findings, we hypothesized that IL-8/STAT3 signaling may be a key molecular mechanism in the cross talk between TME and CSCs. In this study, we assessed macrophage polarization and CSC stemness in SKOV3 cells after addition of rhIL-8 or IL-8 Ab, and detected the effects of STAT3 knockdown or overexpression on macrophage polarization and CSC stemness.

2. Materials and methods

2.1. Cell culture, sphere formation, co-culture experiments

Human ovarian cancer SKOV3 cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin solution, in a humidified environment containing 5% CO_2 at 37 °C.