



英语学习与文献汇报

English learning & Literature reviewing

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Association between cytokines and exosomes in synovial fluid of individuals with knee osteoarthritis

膝关节骨性关节炎患者滑膜液中细胞因子与外泌体的关系



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Introduction

- ▶ Cytokines in synovial fluid (SF) play a crucial role in knee osteoarthritis (KOA). Exosomes are nanovesicles that are abundant in SF and carry a large quantity of signaling molecules. The purpose of this study was to evaluate the cytokine profiles of SF-derived exosomes and try to explore its biological function.
- ▶ 膝关节滑膜液、外泌体、细胞因子与膝骨关节炎的关系
- ▶ Twenty-four KOA patients, PBMCs and chondrocytes



Introduction

- ▶ Our data indicated that most cytokines in SF are not only in a free form but also associated with and enriched in exosomes. Exosomes from end-stage KOA patients has a higher level of cytokines, especially chemokines, in comparison with the cytokine profiles of the soluble SF. SF-derived exosomes recruit inflammatory cells and inhibit cartilage proliferation, thus promoting joint degeneration. These data provide a new perspective for understanding the changes in the inner environment of KOA.
- ▶ 晚期KOA患者的外泌体细胞因子水平较高，尤其是趋化因子。SF来源的外泌体招募炎症细胞，抑制软骨增殖，从而促进关节退行性变。



Exosomes外泌体是什么？

- ▶ Exosomes are 30-120 nm extracellular vesicles released by various cells that transport proteins, DNA, messenger RNAs (mRNAs) and noncoding RNAs (ncRNAs), facilitate intercellular communication and regulate immune responses.
- ▶ Exosomes that exist in various body fluids, including blood, urine, sweat, milk and semen have been widely studied. However, SF-derived exosomes remain poorly characterized.

Materials and Methods

- ▶ 24 Patients
- ▶ Mild (KL1-2) group ($n=12$, 6 men and 6 women, 54.25 ± 8.61 years), representing the early-stage
- ▶ Severe (KL3-4) group ($n=12$, 5 men and 7 women, 59.08 ± 4.64 years), representing the end-stage
- ▶ Collection of Knee Synovial Fluid 2ml
- ▶ Isolation of Exosomes from Synovial Fluid
- ▶ transmission electron microscopy (TEM, 透射电镜)
- ▶ Analysis of the cytokines in SF and purified SF-derived exosomes (细胞因子表达谱)
- ▶ Cell culture (细胞迁移与增殖实验)

Results

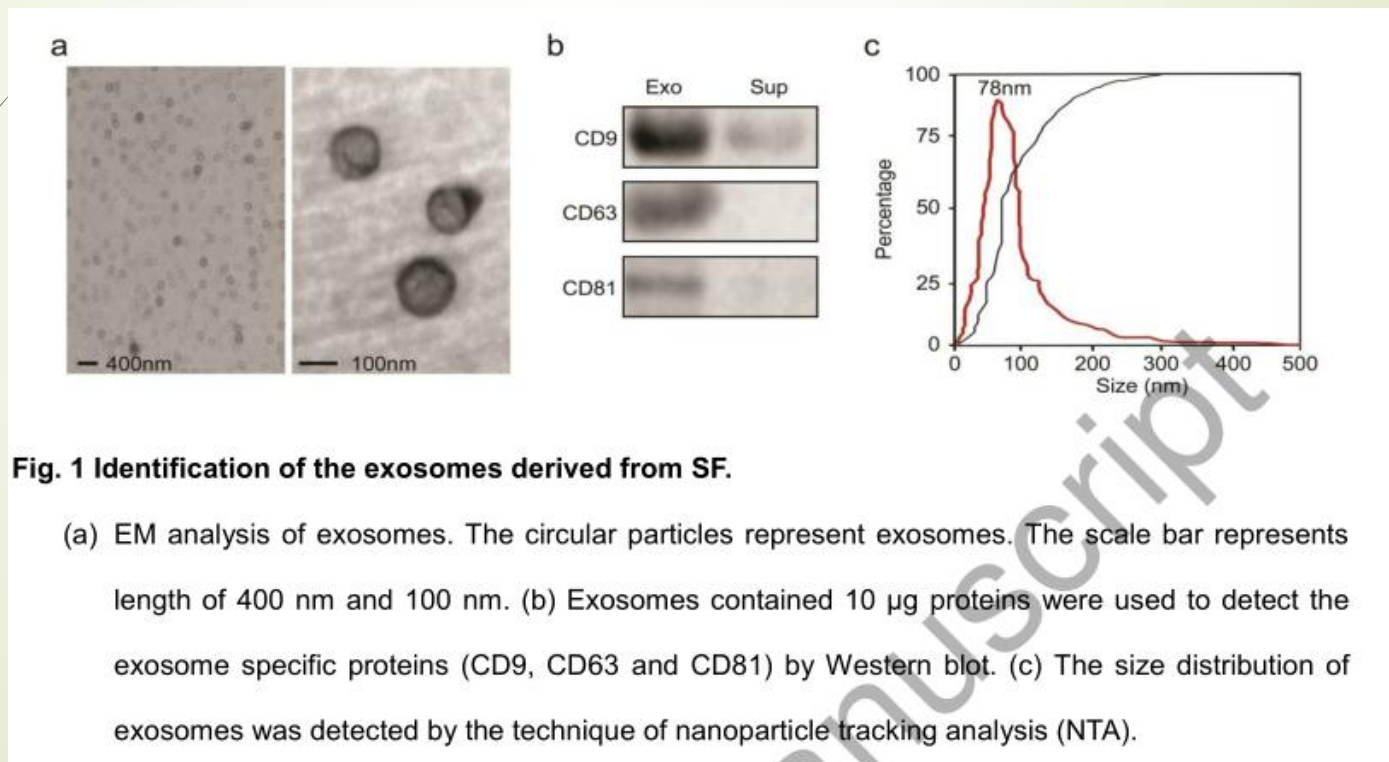
► Patient demographics

Mild group				Severe group			
Patient No.	Gender	Age (Years)	KL	Patient No.	Gender	Age (Years)	KL
1	M	47	2	1	M	58	3
2	M	54	2	2	W	56	3
3	W	65	2	3	W	58	4
4	M	38	1	4	M	65	4
5	W	64	2	5	W	62	3
6	W	55	2	6	M	60	4
7	W	58	1	7	M	59	4
8	M	64	2	8	W	55	4
9	M	62	2	9	W	52	4
10	W	49	2	10	M	54	4
11	W	48	2	11	W	62	4
12	M	47	2	12	W	68	4

Abbreviation: M, male; W, women; KL, Kellgren-Lawrence

Results

- Characterization of exosomes derived from SF



Results

Characterization of exosomes derived from SF

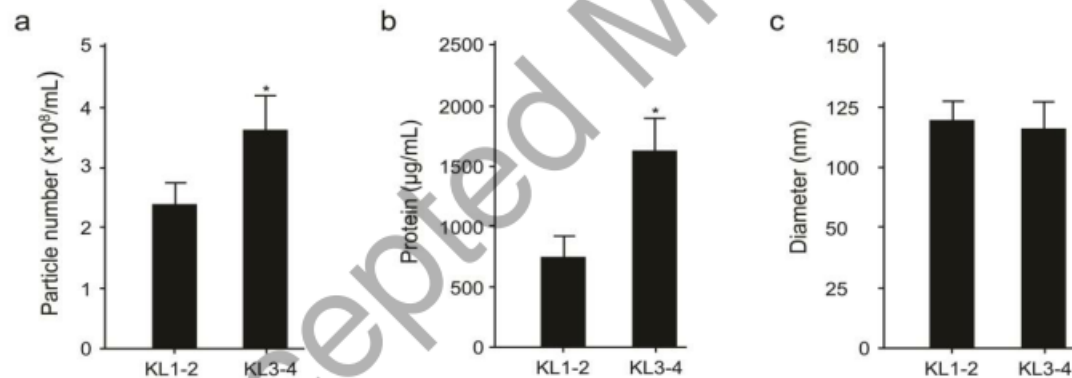



Fig. 2 Characterization of exosomes derived from SF of KOA patients at different stages.

(a) The difference of exosome particle number. The exosomes were separated from 1 ml SF at different stage for the number count with NTA. (b) The difference of exosome protein concentration. The exosomes were separated from 1 ml SF of KOA patients at different stages for the protein quantitation with Bradford assay. (c) Particle size of exosomes was analyzed by the NTA method. Each SF sample represents a synovial fluid mixture from three patients. Data were shown as the mean \pm standard (SD) (n=4) and analyzed by Mann-Whitney U test. * P < 0.05, compared with the KL1-2 group.



Results

- ▶ The expression profiles of inflammatory cytokines in SF and SF-derived exosomes
- ▶ We compared the expression profiles of inflammatory cytokines in SF and SF-derived exosomes of the KL1-2 group with that of the KL3-4 group.
- ▶ The expression profiles of chemokines in SF and SF-derived exosomes
- ▶ Next, we analyzed the expression profiles of the chemokines.

Results

Table 2 The expression profiles of cytokines in the SF and SF-derived exosomes

Cytokines	Exosome cytokine (pg/mL), median (IQR)		P Value	SF cytokine (pg/mL), median (IQR)		P value
	KL1-2	KL3-4		KL1-2	KL3-4	
IL-1 α	14.56 (12.48-15.66)	34.31 (31.24-34.99)	0.021*	41.19 (33.50-50.89)	49.56 (45.22-58.61)	0.343
IL-1 β	23.90 (22.76-28.06)	154.05 (129.36-195.36)	0.010*	50.44 (35.46-65.28)	183.76 (143.53-213.56)	0.029*
IL-2	18.52 (17.65-19.96)	67.88 (57.85-76.50)	0.020*	45.23 (32.65-64.05)	85.16 (63.00-106.26)	0.114
IL-4	0 (0-0.03)	13.29 (10.04-18.79)	0.018*	154.23 (130.87-164.00)	152.16 (141.92-182.13)	1
IL-5	undetected	46.99 (29.54-66.22)	N/A	27.55 (23.33-32.87)	51.87 (42.77-68.96)	0.057
IL-6	4.75 (2.07-8.47)	59.17 (49.41-65.46)	0.020*	71.64 (63.31-77.32)	85.93 (67.79-98.30)	0.486
IL-10	25.29 (22.91-31.04)	29.81 (21.03-37.64)	0.773	249.96 (226.55-257.16)	120.28 (95.49-149.40)	0.029*
IL-12p70	39.05 (28.78-49.20)	128.33 (113.63-146.60)	0.011*	74.50 (63.71-88.98)	99.82 (87.44-104.81)	0.686
IL-13	undetected	20.76 (16.78-23.29)	N/A	72.96 (56.13-93.24)	92.82 (77.10-106.01)	0.486
IL-15	3.53 (1.17-6.81)	15.61 (13.91-18.07)	0.043*	164.24 (149.12-188.93)	47.14 (37.17-58.14)	0.029*
IL-17	3.02 (2.47-4.02)	35.27 (33.46-37.86)	0.029*	45.48 (39.98-49.23)	171.00 (147.15-191.87)	0.021*
TNF- α	39.68 (32.00-45.46)	114.98 (102.06-133.96)	0.026*	254.60 (209.02-286.89)	279.67 (218.18-322.91)	0.316
IFN- γ	16.73 (14.53-18.68)	66.79 (56.89-79.05)	0.020*	190.45 (182.75-206.38)	411.76 (395.41-432.60)	0.018*
CCL2	5.93 (4.56-6.82)	88.68 (75.86-98.60)	0.021*	139.50 (126.76-145.67)	190.20 (154.97-223.07)	0.20
CCL3	16.28 (15.13-17.02)	150.98 (140.13-168.09)	0.018*	41.04 (34.80-48.39)	45.20 (26.24-64.64)	1
CCL5	undetected	176.48 (172.90-196.08)	N/A	63.24 (39.46-86.85)	250.48 (210.61-272.29)	0.029*
CCL15	0.19 (0-0.495)	58.03 (36.74-76.24)	0.020*	150.58 (119.16-160.10)	257.60 (218.26-281.65)	0.567
CXCL8	7.35 (4.92-10.44)	51.74 (37.37-70.03)	0.019*	50.10 (46.16-56.77)	141.40 (124.56-151.72)	0.021*
CXCL9	6.17 (3.46-8.98)	89.28 (88.19-94.07)	0.029*	161.82 (155.66-170.52)	298.70 (210.64-421.76)	0.240
CXCL12	13.57 (9.81-16.85)	147.97 (121.18-187.88)	0.000*	59.96 (55.96-64.39)	125.51 (100.88-141.98)	0.686
G-CSF	51.02 (39.02-56.71)	86.66 (79.23-89.32)	0.029*	146.22 (143.31-154.78)	296.55 (242.38-357.20)	0.343

Results

- ▶ SF-derived exosomes activate PBMC chemotaxis and inhibit chondrocyte proliferation

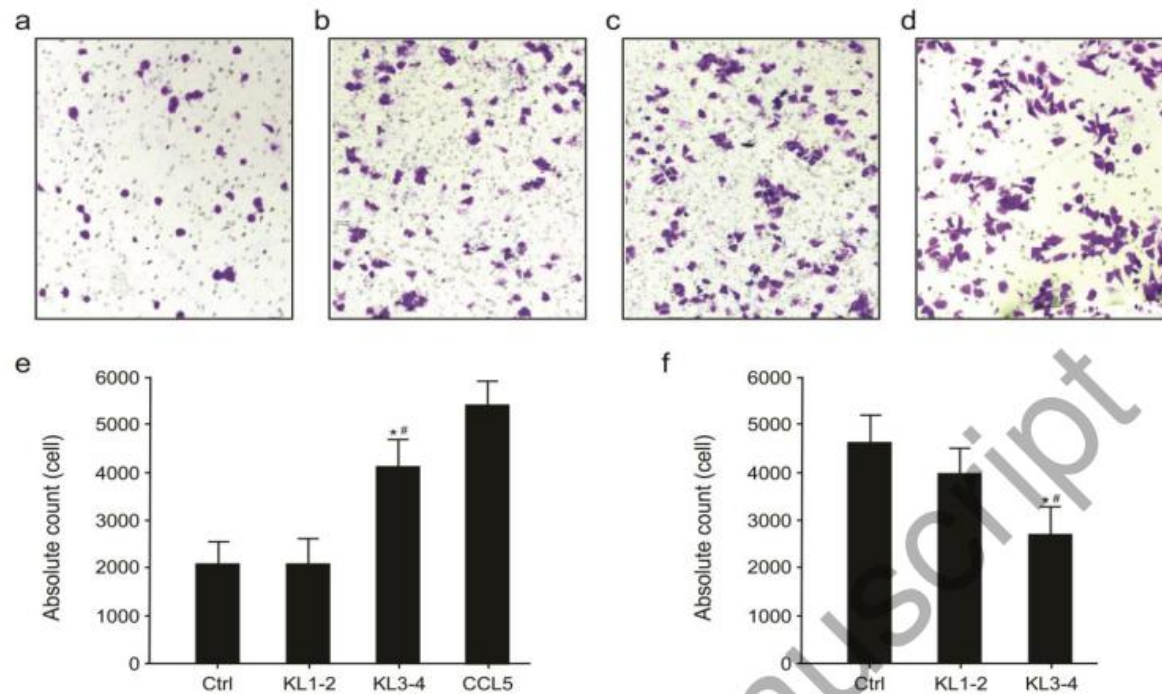


Fig.3 SF-derived exosomes enhance chemotaxis of PBMCs and inhibit chondrocyte proliferation.



Discussion

- ▶ As far as we know, this is the first report to extensively evaluate cytokine levels in exosomes derived from the SF of KOA patients.
- ▶ Previous studies have displayed the free form cytokine profiles in KOA SF;
- ▶ however, the forms resulting from the combination of cytokines and exosomes have not been studied.



Discussion

- ▶ Along with the development of molecular biology, an increasing number of studies have shown that various cytokines play important roles in the development of KOA.
- ▶ Exosomes are extracellular vesicles released by various cells with the capability of transporting signaling molecules.
- ▶ In this report, we extracted exosomes from SF and detected 21 cytokines in SF and SF derived-exosomes at different stages of KOA.



Discussion

- ▶ Chemokines are small secretory proteins that are involved in inflammatory and immune responses.
- ▶ Unlike the cytokine profile in synovial fluid, chemokines levels were significantly higher in end-stage KOA SF-derived exosomes.
- ▶ CCL2, CCL3, CCL15, CXCL8, CXCL9 and CXCL10 levels were low in early-stage KOA SF-derived exosomes, while CCL5 and CCL8 were undetectable.



Discussion

- ▶ In conclusion, this study demonstrated that most cytokines/chemokines in knee SF exist not only in the free soluble form but also in an insoluble form combined with exosomes. Moreover, exosomes derived from SF of end-stage KOA enhanced PBMC chemotaxis and inhibited chondrocyte proliferation, thus contributing to joint inflammation and cartilage degeneration, resulting the increased severity of arthritis.
- ▶ Characterizing the role of KOA SF-derived exosomes will strengthen our understanding of the mechanisms of KOA development and provide potential targets for therapeutic applications.



Discussion

- 单中心，病例数量较少，滑膜液量受限
- 无健康人群对照数据，只有KOA轻重对比
- 患者软骨细胞培养，自身退化影响结果
- 进一步的机制研究
- 体内动物实验

- 临床样本→表达谱分析→简单验证



THANK YOU.

感谢各位老师、师兄
弟的收听，欢迎提问！