International Journal of Gerontology xxx (2018) 1–6

Contents lists available at ScienceDirect

International Journal of Gerontology

journal homepage: www.ijge-online.com

Original Article

The Comparison of Exosome and Exosomal Cytokines between Young and Old Individuals with or without Gastric Cancer

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ARTICLE INFO

Article history:
Received 5 June 2017
Received in revised form
23 February 2018
Accepted 15 March 2018
Available online xxx

Keywords:
exosome,
microvesicle,
aging,
cancer,
cytokine

SUMMARY

Background: Exosomes are small extracellular vesicles secreted by various types of cells. Exosomes play an important role in intercellular communication by serving as vehicles for transferring proteins, lipids, mRNAs, microRNAs, and DNAs to recipient cells. This study investigated the role of exosomes in gastric cancer and the aging process by using a large number of human serum samples from young and aged individuals with or without gastric cancer.

Methods: The age- and cancer-associated changes in exosome levels and exosomal cytokine content were measured. Exosomes in human serum were isolated by using a total exosome isolation reagent, followed by a quantification of isolated exosomes using a MicroBCA assay. Exosomal cytokines were measured by ELISA.

Results: Serum exosome levels are increased by cancer and aging. Exosomal levels of TNF-α and TGF-β are increased, whereas IL-10 levels are reduced in gastric cancer. In addition, the cancer-associated changes in exosomal cytokines remain constant with age, even though aging affects every type of immune cells and immunomodulating factors.

Conclusion: Gastric cancer and aging affect serum exosome and exosomal cytokines levels. Future studies would require the analysis of more diverse cytokines in exosomes derived from multiple types of cancer.

1. Introduction

Exosomes are small extracellular vesicles of 30–100 nm in diameter that are secreted by various types of cells. They can be detected in body fluids such as urine, serum, saliva, and breast milk as well as cell culture media. Initially, exosomes were thought to function in waste disposal, to eliminate unnecessary cellular components. However, studies have revealed that they play an important role in intercellular communication by serving as vehicles for transferring proteins, lipids, mRNAs, microRNAs, and DNAs to recipient cells. Because exosomes are of endocytic origin, they commonly contain endosome-associated proteins including Rab GTPase, SNARE, annexin, and tetraspanins such as CD63, CD81, CD82, CD53, and CD37. In addition, the content of exosomes varies with cell origins and the physiological and pathological conditions of exosome-secreting cells. For example, exosomes released by platelets contain a tissue factor involved in coagulation,[1] whereas those from antigen presenting cells contain major histocompatibility complex class II[2]. Tumor-derived exosomes harbor various cellular factors promoting tumor-growth, angiogenesis, invasion, metastasis, immunosuppression, or drug resistance.[3,4] Because exosomes are highly associated with disease initiation and progression, understanding the diverse exosomal contents and their specialized functions can provide new diagnostic and therapeutic tools for disease control.

During aging, the human body undergoes changes in appearance and declines in physiological function, increasing the chances of developing numerous diseases.[5,6] Aging is largely driven by changes in protein production and turnover, and affects individual body systems to different degrees in an organ-specific manner.[7,8]

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https://doi.org/10.1016/j.ijge.2018.03.013

Exosomes have also been found to play a role in cellular senescence.[9] Some studies have reported that senescent cells produce high levels of exosomes, and these senescence-associated exosomes contain immunoregulatory factors and microRNAs that contribute to influence aging process.[10,11] Aging is an important risk factor for cancer, and cancer incidence increases with age. Studies have shown that exosomes are involved in cancer development,[12] progression,[13] severity, and response to treatment by transferring proteins and nucleic acids, including microRNAs,[14] to recipient cells in the tumor microenvironment or in distant regions.[15] However, it has not been studied whether exosomes affect cancer differently in young and aged individuals.

Therefore, in the current study, we examined the age- and cancer-associated changes in exosome and exosomal cytokine content using a large number of human serum samples from healthy population and gastric cancer patients, both including young and old subgroup, resulting in the analysis of 4 subgroups.

2. Materials and methods

2.1. Human serum samples

Human serum samples were kindly provided by Prof. Sun Young Rha (Yonsei University College of Medicine, Seoul, Korea). The serum samples were obtained from 69 young (median age 26 years, range 23–45) and 80 old (median age 75 years, range 63–90) healthy individuals, and 66 young (median age 39 years, range 26–45) and 70 old (median age 72 years, range 65–86) patients with gastric cancer of stages I through IV (Table 1). The patient samples were taken from patients who were diagnosed with gastric cancer and underwent cancer treatment at Yonsei Cancer Center, Severance Hospital (Yonsei University Health System, Seoul, Korea). The normal serum samples were obtained from healthy individuals who visited Yonsei Severanc Hospital for regular health exams and were free from cancer and chronic disease including diabetes mellitus, cystic fibrosis, asthma, immunological disorder, and COPD. Serum samples were prepared by centrifuging tubes with whole blood at 2000 g for 10 min at 4 °C to remove clot after coagulation. The supernatants were carefully removed and stored at −70 °C until analyzed. This study was approved by the Ethics Committee in Yonsei Cancer Center, Yonsei University College of Medicine.

Overall survival (OS) was calculated from the date of gastrectomy to the date of death, while recurrence-free survival (RFS) was defined as the interval between the date of gastrectomy and the date of either recurrence or death.

2.2. Isolation and quantification of exosomes

Exosomes in human serum were isolated by using a total exosome isolation reagent (Invitrogen, Carlsbad, CA, USA), following manufacturer's instructions. Briefly, human serum was treated with 1/5 of isolation agent and incubated at −20 °C over 15 h. After incubation, the mixture was centrifuged at 10000 × *g* for 30 min, and the supernatant was removed. To obtain exosomes with high purity, the pellet was suspended twice in sterilized PBS. For quantification of isolated exosomes, a MicroBCA assay (Thermo Fischer Scientific, Waltham, MA, USA) was performed according to the manufacturer's instructions. The absorbance was then measured at 540 nm using a ThermoMax plate Reader (Molecular Devices, Sunnyvale, CA, USA).

Table 1
Demographic data of young/old gastric cancer patients.

Variables		Total		YOUNG		OLD	
		Total N	N = 136 %	Total N	N = 66 %	Total N	N = 70 %
Age	Median±S.D. (min-max)	65 ± 17.5 (25-86)		39 ± 4.9 (25-45)		72 ± 5 (65-86)	
Sex	Male	85	62.5	33	50.0	52	74.3
	Female	51	37.5	33	50.0	18	25.7
Operation	Yes	102	75.0	48	72.7	54	77.1
	No	34	25.0	18	27.3	16	22.9
Stage	I	42	30.9	22	33.3	20	28.6
	II	17	12.5	7	10.6	10	14.3
	III	37	27.2	17	25.8	20	28.6
	IV	40	29.4	20	30.3	20	28.6
Histology	AWD	15	11.0	2	3.0	13	18.6
	AMD	31	22.8	5	7.6	26	37.1
	APD	62	45.6	35	53.0	27	38.6
	SRC	24	17.6	23	34.8	1	1.4
	Others	4	2.9	1	1.5	3	4.3
Lauren classification	Intestinal	40	29.4	9	13.6	31	44.3
	Diffuse	40	29.4	28	42.4	12	17.1
	Mixed	5	3.7	2	3.0	3	4.3
	Unknown	51	37.5	27	40.9	24	34.3
HER2	Positive	11	8.1	3	4.5	8	11.4
	Negative	70	51.5	34	51.5	36	51.4
	Unknown	55	40.4	29	43.9	26	37.1
Recurrence	Yes	25	18.4	12	18.2	13	18.6
	No	111	81.6	54	81.8	57	81.4
Survival	Death	60	44.1	28	42.4	32	45.7
	Alive	76	55.9	38	57.6	38	54.3

AWD, well differentiated adenocarcinoma; AMD, moderate differentiated adenocarcinoma; APD, poorly differentiated adenocarcinoma; SRC, signet ring cell carcinoma. Several pathologic factors, including tumor histology, tumor type by Lauren classification, and pathologic TNM staging according to the 7th American Joint Committee on Cancer criteria were obtained from the slide review by two individual pathologists at Yonsei Cancer Center. Tumor histology was classified based on Japanese gastric cancer treatment guidelines 2010. HER2 positivity was defined using the HER2 scoring criteria for gastric cancers by Hofmann et al. (Histopathology 52(7):797), and was confirmed using immunohistochemical staining (IHC; Hercep Test, Dako, Denmark) or silver **in situ** hybridization (SISH; Ventana Discovery XT system, Ventana/Roche, USA).

Please cite this article in press as: Im K, et al., The Comparison of Exosome and Exosomal Cytokines between Young and Old Individuals with or without Gastric Cancer, International Journal of Gerontology (2018), https://doi.org/10.1016/j.ijge.2018.03.013