World Journal of Otorhinolaryngology-Head and Neck Surgery (2018) 4, 175—178

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/WJOHNS; www.wjent.org

Review Article

Exosomes and chronic rhinosinusitis

Sarina K. Mueller [a,b]

[a] Friedrich-Alexander University Erlangen-Nürnberg (FAU), Department of Otorhinolaryngology, Head and Neck Surgery, Waldstrasse 1, 91054, Erlangen, Germany
[b] Massachusetts Eye and Ear Infirmary, Harvard Medical School, 243 Charles Street, 02114, Boston, USA

Received 12 July 2018; accepted 17 July 2018
Available online 1 November 2018

Abstract The objective of this manuscripts to review current knowledge regarding exosomes as they relate to the physiology and pathology of the human nose as well as their role as biomarkers of chronic rhinosinusitis with nasal polyps (CRSwNP).

Exosomes are 30—150 nm membrane-bound vesicles secreted by virtually all cell types. Exosomes contribute to the rapid inter-epithelial transfer of proteins and mediate innate immunosurveillance and defense mechanisms in the human nasal cavity. Exosomes also protect their cell specific cargo from degradation by nucleases and proteases and mirrorCRS related tissue protein perturbations more effectively than whole mucus. Thus, exosomal isolation and analysis may be used to non-invasively monitor disease severity, prognosis, and potentially even treatment response.

Recent studies of exosomes in CRS suggest they can be used to study the immunopathology of chronic sinonasal inflammation. Furthermore, their relative accessibility suggests that exosomal proteomescan be used as non-invasive, serial, and quantitative biosignatures for rhinosinusitis that can be sampled in clinic in order to predict disease severity, prognosis, and treatment response. Exosomal research has also led to important revelations regarding their physiologic function as they seem to play an important role in innate immunosurveillance and defense. However, exosomal research is still nascent and cost-effectiveness as well as feasibility of implementation in the routine workup for CRS have to be further explored.

E-mail address: mueller.sarinakatrin@gmail.com.
Peer review under responsibility of Chinese Medical Association.

Production and Hosting by Elsevier on behalf of KeAi

Introduction

Chronic rhinosinusitis without (CRSsNP) and with nasal polyps (CRSwNP) represent two different endotypes. CRSwNPis a chronic inflammatory mucosal disease of the nose and sinuses. It is moderately common within the population with a prevalence of 2%—5%[1—3] and occurs

https://doi.org/10.1016/j.wjorl.2018.07.005

mainly after the age of 30.[4] The prevalence increases with age (5% at 60 years of age and older) and the annual direct cost for CRS is estimated to be approximately $10 billion.[5–11]Associations between CRSwNP and ciliary impairment,[12] asthma,[13,14] aspirin sensitivity[4] and immunocompromised state[15,16] have been described. Furthermore, exogenic (chemical-physical or infectious) as well as endogenic (genetic, hormonal, immunological and metabolic) factors have been explored.[15,17–26] Histologically eosinophilic infiltration of the mucosa and secretions can be detected.[27,28] The degree of tissue eosinophilia correlates with objective disease severity and co-morbid asthma.[5,7,21,29,30] Despite recent advances in our understanding of CRSwNP, the etiopathology of the disease state remains unclear. Consequently, optimal diagnostic and treatment algorithms remain elusive as no single accurate biosignature of CRSwNP has yet been developed.[21]

Exosomes are spherical to cup-shaped, 30–150 nm[31,32]membrane-bound vesicles. They consist of a lipid bilayer derived from their host cell and incorporate proteins, lipids, DNA and RNA that are specific to their cell of origin.[33–35] Exosomes are secreted by virtually all cell types into almost all body fluids including nasal mucus.[36] Due to their ubiquitous presence, their specificity to their cell of origin, and their easy accessibility, exosomes exhibit great potential for non-invasive diagnostics.[37] Biomarkers may be defined as biological substances that are specific and sensitive to a particular physiological or pathological condition. Because exosomes are cell specific and reflective of particular cell functions, they may be used to develop novel biomarkers for clinical diagnosis. Moreover, exosomes protect their cargo from degradation by nucleases and proteases thereby increasing biomarker half-life which can serve to facilitate downstream molecular analyses.[38] The objective of this article is to therefore review the current knowledge regarding the role of exosomes as contributors to both health and disease and as sources of diagnostic material in CRSwNP.

Physiologic and pathophysiologic function of exosomes in the human nasal cavity

There are several hypotheses regarding the physiologic function of exosomes. Generally, exosomes are thought to act as intercellular shuttles by transferring functional nucleic acids and proteins between cells.[35,39] The nasal cavity is a mucosal barrier that represents one of the first sites of contact between the environment and the body. As a consequence, this barrier harbors a variety of protective mechanisms to defend against pathogens. Amongst those, mucociliary clearance (MCC)[40] and epithelial expression of toll-like receptors (TLRs) have been described. The stimulation of TLRs by microbial structural motifs known as pathogen-associated molecular patterns (PAMPs)[41,42] has been shown to release antimicrobial peptides (AMPs)[43–45] into the nasal mucus (unpublished data). Early evidence suggests that nasal mucosa derived exosomes (NMDEs)may transport these AMPs directly to the surface of the pathogen thereby inducing direct microbiocidal effects as well as donating these AMPs to adjacent, pathogen naive, epithelia (unpublished data). Finally, NMDEs are able to promote the migration of several immune cells in vitro including monocytes, neutrophils and NK cells further contributing to mucosal immune defense.[46]

Beyond their innate immune physiologic activity, NMDEs are also believed to play a role in the spread of pathologies such as cancer or inflammatory disease via autocrine, endocrine and paracrine pathways of secretion as well as through mucociliary clearance.[47] Exosomal P-glycoprotein (P-gp), an efflux pump which drives type-2 helper T-cell inflammation in CRSwNP, has been shown to be present and significantly enriched in CRSwNP exosomes relative to controls.[47,48] High P-gp secretion has also been associated with worse subjective as well as objective measures of disease severity.[48]Previous studies have shown that NMDEs are capable of the rapid inter-epithelial transfer of pro-inflammatory proteins, including P-gp, which function to propagate inflammation in the setting of CRS and create a field inflammatory effect.[47]

Exosomes as noninvasive biomarkers in CRS

The development of a reproducible, non-invasive, serial, and quantitative "liquid biopsy" for rhinosinusitis represents a holy grail in rhinology. Ideally, these biomarkers would mirror the sensitivity and specificity of a tissue biopsy while allowing non-invasive outpatient sampling. This would also enable prospective, serial testing in clinic for diagnostic and prognostic purposes. In the comparison between whole mucus and exosomes isolated from whole nasal mucus, the proteomic perturbations indicative of CRSwNP within tissue are highly reflected in the exosomal population leading to a 20-fold increase in the number of overlapping differentially regulated proteins within exosomes as compared to whole mucus. Additionally, inter-patient variance is significantly lower in the exosomes than in matched whole mucus samples (unpublished data). To increase reproducibility and specificity of the "liquid biopsy", a high signal-to-noise ratio is crucial. Exosomes represent encapsulated proteins, lipids, DNA and RNA from the cell of origin whereas whole mucus represents a heterogenous mixture of cellular debris, cytokines and degraded proteins fragments. Furthermore, the exosomal proteome is protected from degradation by nucleases and proteases there by contributing to its stability as a biomarker substrate.

Historically, two major challenges with exosomal proteomic work have been described. The first has been the low sample volume available after ultracentrifugation dependent purification. However, there are techniques including SOMAscan (SomaLogic), a highly multiplexed, sensitive, and quantitative immune-like proteomic tool, which are capable of handling the small sample size and a 12–13 log difference in expression.[59] The second challenge lies in the relatively easy but long and expensive exosome isolation. The most common technique for concentrating exosomes is ultracentrifugation. Other techniques include immunoaffinity-based isolation techniques, commercial precipitation kits, acoustic nano-filter systems and the nanowire-on-micropillar technique. Optimization and validation of the most effective methods remain a barrier to the adoption of exosomal analysis in routine clinical practice.[50]

As previously noted, exosomal P-gp represents a non-invasive biomarker that can be used to predict pathological patterns and disease severity. For this reason, P-gp has also been identified as a potential drug target for treating CRSwNP. A recent double-blind placebo-controlled randomized clinical trial demonstrated that verapamil, a well-known and tolerated inhibitor of P-gp, was capable of reducing both subjective and objective measures of CRSwNP.[51] Other exosomal biomarkers which have been identified in CRS include proteins of the fibrinolysis coagulation cascades. The fibrinolysis pathway was shown to be down regulated whereas the coagulation cascade was upregulated (unpublished data). These findings lend further support to the hypothesis that proteolytic imbalances may play an important role in the etiopathogenesis of CRSwNP.[52,53] Due to the reproducible protein expression pattern, the exosomal proteins of these pathways could potentially be utilized as a disease biosignature.

Conclusion

Recent studies of exosomes in CRS have suggested that they not only participate in disease pathogenesis but may also be used as a substrate for stable, non-invasive biomarkers of disease. Simultaneously, this research has led to important revelations about the physiologic function of exosomes as they seem to play an important role ininnate nasal immunosurveillance and antimicrobial defense.

Conflict of interest

None.

Financial disclosures

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Giannoni CM, Stewart MG, Alford EL. Intracranial complications of sinusitis. *Laryngoscope*. 1997;107:863—867.
2. Hedman J, Kaprio J, Poussa T, Nieminen MM. Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. *Int J Epidemiol*. 1999;28:717—722.
3. Klossek JM, Neukirch F, Pribil C, et al. Prevalence of nasal polyposis in France: a cross-sectional, case-control study. *Allergy*. 2005;60:233—237.
4. Szczeklik A, Nizankowska E, Sanak M, Swierczynska M. Aspirin-induced rhinitis and asthma. *Curr Opin Allergy Clin Immunol*. 2001;1:27—33.
5. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol*. 2001;107:607—614.
6. Orlandi RR, Kingdom TT, Hwang PH, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6(Suppl 1):S22—S209.
7. Bhattacharyya N, Vyas DK, Fechner FP, Gliklich RE, Metson R. Tissue eosinophilia in chronic sinusitis: quantification techniques. *Arch Otolaryngol Head Neck Surg*. 2001;127:1102—1105.
8. Rosenfeld RM, Andes D, Bhattacharyya N, et al. Clinical practice guideline: adult sinusitis. *Otolaryngol Head Neck Surg*. 2007;137:S1—S31.
9. Scangas GA, Remenschneider AK, Su BM, Shrime MG, Metson R. Cost utility analysis of endoscopic sinus surgery for chronic rhinosinusitis with and without nasal polyposis. *Laryngoscope*. 2017;127(1):29—37.
10. Bhattacharyya N. Incremental health care utilization and expenditures for chronic rhinosinusitis in the United States. *Ann Otol Rhinol Laryngol*. 2011;120:423—427.
11. Caulley L, Thavorn K, Rudmik L, Cameron C, Kilty SJ. Direct costs of adult chronic rhinosinusitis by using 4 methods of estimation: Results of the US Medical Expenditure Panel Survey. *J Allergy Clin Immunol*. 2015;136:1517—1522.
12. Al-Rawi MM, Edelstein DR, Erlandson RA. Changes in nasal epithelium in patients with severe chronic sinusitis: a clinicopathologic and electron microscopic study. *Laryngoscope*. 1998;108:1816—1823.
13. Salvin RG, Cannon RE, Friedman WH, Palitang E, Sundaram M. Sinusitis and bronchial asthma. *J Allergy Clin Immunol*. 1980;66:250—257.
14. Schwartz HJ, Thompson JS, Sher TH, Ross RJ. Occult sinus abnormalities in the asthmatic patient. *Arch Intern Med*. 1987;147:2194—2196.
15. Chee L, Graham SM, Carothers DG, Ballas ZK. Immune dysfunction in refractory sinusitis in a tertiary care setting. *Laryngoscope*. 2001;111:233—235.
16. Porter JP, Patel AA, Dewey CM, Stewart MG. Prevalence of sinonasal symptoms in patients with HIV infection. 1999;13:203—208.
17. DeMarcantonio MA, Han JK. Nasal polyps: pathogenesis and treatment implications. *Otolaryngol Clin North Am*. 2011;44:685—695.
18. Zhang XH, Lu X, Long XB, et al. Chronic rhinosinusitis with and without nasal polyps is associated with decreased expression of glucocorticoid-induced leucine zipper. *Clin Exp Allergy*. 2009;39:647—654.
19. Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108:S147—S334.
20. Settipane GA. Epidemiology of nasal polyps. *Allergy Asthma Proc*. 1996;17:231—236.
21. Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinol Suppl*. 2012;23:1—298, 3 p preceding table of contents.
22. Hosemann W, Weber RK, Keerl RE. *Minimally invasive endonasal sinus surgery*. New York Stuttgart: Thieme Press; 2000.
23. Caplin I, Haynes JT, Spahn J. Are nasal polyps an allergic phenomenon. *Ann Allergy*. 1971;29:631—634.
24. Sun Y, Zhou B, Wang CS, et al. Clinical and histopathologic features of biofilmassociated chronic rhinosinusitis with nasal polyps in Chinese patients. *Chin Med J (Engl)*. 2012;125:1104—1109.
25. Zinreich SJ, Mattox DE, Kennedy DW, Chisholm HL, Diffley DM, Rosenbaum AE. Concha bullosa: CT evaluation. *J Comput Assist Tomogr*. 1988;12:778—784.
26. Cohen M, Kofonow J, Nayak JV, et al. Biofilms in chronic rhinosinusitis: a review. *Am J Rhinol Allergy*. 2009;23:255—260.
27. Kaliner MA, Osguthorpe JD, Fireman P, et al. Sinusitis: bench to bedside. Current findings, future directions. *J Allergy Clin Immunol*. 1997;99:S829—S848.
28. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol*. 1988;81:867—875.
29. Szucs E, Ravandi S, Goossens A, Beel M, Clement PA. Eosinophilia in the ethmoid mucosa and its relationship to the

severity of inflammation in chronic rhinosinusitis. *Am J Rhinol*. 2002;16:131—134.
30. Soler ZM, Sauer DA, Mace J, Smith TL. Relationship between clinical measures and histopathologic findings in chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2009;141:454—461.
31. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013;200:373—383.
32. Simons M, Raposo G. Exosomes–vesicular carriers for intercellular communication. *Curr Opin Cell Biol*. 2009;21:575—581.
33. Pisitkun T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. *Mol Cell Proteomics*. 2006;5:1760—1771.
34. Qin J, Xu Q. Functions and applications of exosomes. *Acta Pol Pharm*. 2014;71:537—543.
35. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9:654—659.
36. Wu G, Yang G, Zhang R, et al. Altered microRNA Expression Profiles of Extracellular Vesicles in Nasal Mucus From Patients With Allergic Rhinitis. *Allergy Asthma Immunol Res*. 2015;7:449—457.
37. Crowley E, Di NF, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol*. 2013; 10:472—484.
38. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol*. 2008;110:13—21.
39. Thébaud B, Stewart DJ. Exosomes: cell garbage can, therapeutic carrier, or trojan horse. *Circulation*. 2012;126: 2553—2555.
40. Antunes MB, Gudis DA, Cohen NA. Epithelium, cilia, and mucus: their importance in chronic rhinosinusitis. *Immunol Allergy Clin North Am*. 2009;29:631—643.
41. Kato A, Schleimer RP. Beyond inflammation: airway epithelial cells are at the interface of innate and adaptive immunity. *Curr Opin Immunol*. 2007;19:711—720.
42. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine*. 2008;42:145—151.
43. Skerrett SJ, Braff MH, Liggitt HD, Rubens CE. Toll-like receptor 2 has a prominent but nonessential role in innate immunity to Staphylococcus aureus pneumonia. *Physiol Rep*. 2017;5. pii: e13491.
44. Kvarnhammar AM, Rydberg C, Järnkrants M, et al. Diminished levels of nasal S100A7 (psoriasin) in seasonal allergic rhinitis: an effect mediated by Th2 cytokines. *Respir Res*. 2012;13:2.
45. Martínez-Castillo M, Santos-Argumedo L, Galván-Moroyoqui JM, Serrano-Luna J, Shibayama M. Toll-like receptors participate in Naegleria fowleri recognition. *Parasitol Res*. 2018;117(1): 75—87.
46. Lässer C, O'Neil SE, Shelke GV, et al. Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation. *J Transl Med*. 2016;14:181.
47. Nocera AL, Miyake MM, Seifert P, Han X, Bleier BS. Exosomes mediate interepithelial transfer of functional P-glycoprotein in chronic rhinosinusitis with nasal polyps. *Laryngoscope*. 2017; 127:E295—E300.
48. Nocera AL, Meurer AT, Miyake MM, Sadow PM, Han X, Bleier BS. Secreted P-glycoprotein is a noninvasive biomarker of chronic rhinosinusitis. *Laryngoscope*. 2017;127:E1—E4.
49. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics*. 2002;1:845—867.
50. Mueller SK, Nocera AL, Bleier BS. Exosome function in aerodigestive mucosa. *Nanomedicine*. 2018;14:269—277.
51. Miyake MM, Nocera A, Levesque P, et al. Double-blind placebo-controlled randomized clinical trial of verapamil for chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2017; 140:271—273.
52. Kouzaki H, Matsumoto K, Kikuoka H, et al. Endogenous Protease Inhibitors in Airway Epithelial Cells Contribute to Eosinophilic Chronic Rhinosinusitis. *Am J Respir Crit Care Med*. 2017; 195:737—747.
53. Pfeffer PE, Corrigan CJ. An Imbalance between Proteases and Endogenous Protease Inhibitors in Eosinophilic Airway Disease. *Am J Respir Crit Care Med*. 2017;195:707—708.

Edited by Yi Fang