Exosomal ncRNAs

Subjects: Immunology

Contributor: Peter Gahan, Heidi Schwarzenbach

Exosomes, small extracellular vesicles mediate intercellular communication by transferring their cargo including DNA, RNA, proteins and lipids from cell to cell. ExVs contain varying amounts of RNAs concerning over a dozen different RNA forms , the majority of which are classified as ncRNAs (non-coding RNAs).

Keywords: exosomes ; miRNA ; lncRNA ; shRNA ; immune cells ; immune therapy

1. Exosome Biogenesis and Function

ExV formation occurs within the lysosomal system and specifically within multivesicular bodies (MVBs). MVBs develop from late endosomes through membrane in-folding to form small vesicles within, known as intra-vesicular vesicles (IVLs), so leading to the whole structure becoming an MVB. The ExVs are formed via a variety of mechanisms with either one or all mechanisms occurring in the same cell. The best-studied method is one in which MVB formation is coordinated by ESCRT (endosomal sorting complex required for transport). This is comprised of four soluble multi-protein complexes, namely ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III. Usually, they are associated with the cytosolic side of the endosomal membrane so that particular proteins are sorted into the ILVs [1]. A second approach involves ExV formation in the absence of ESCRT[2]. The precise mechanism for ExV release from the MVBs at the plasma membrane has yet to be fully established. Similarly, three possible mechanisms for miRNA uptake from ExVs have been suggested[3][4]. These involve either membrane fusion followed by release of RNA/DNA or macro-pinocytosis or receptor/raft-mediated endocytosis.

Thus, ExVs are viewed as an important subgroup of EVs, moving material and informative molecules between cells and over varying distances. Given the wide range of molecules present, it is clear that, the recipient cells can have their biology modified [5].

ExVs contain a variety of molecules including both genomic and mitochondrial DNA (100 bp–17 kb). Emphasis has been placed on ExV DNA concerning the identification of the presence of a particular cancer and the timing of the appearance of metastases via the liquid biopsy procedure[6][7]. However, it can also be observed that the movement of DNA from cancer to healthy recipient cells can influence the biology of the recipient cells leading to tumor formation [5]. Nevertheless, the literature on the estimation of the DNA content of ExVs is somewhat confusing in that the presence of 93% of the DNA is reorted to be present in ExVs, the remaining DNA being present in the plasma and often being found attached to the outer surface of the ExVs. In contrast, it was shown that there no DNA is present in ExVs, but is present in the larger EVs emanating from tumor cells[8][9].

Additional and important components of ExVs are RNAs, including mRNAs, rRNAs (ribosomal RNAs, tRNAs (transfer RNAs), miRNAs (microRNAs), shRNAs (short hairpin RNAs), lncRNA (long noncoding RNAs), piRNA (piwi-interacting RNAs), snRNA (small nuclear RNAs), and proteins, including ceramides and cholesterol[10]. The mitochondrial DNA and RNAs are present together with mitochondrial proteins that can count for as much as 10% of total ExV proteins[11]. The latter can include the electron transport system as well as complete mitochondria [12]. The ExV RNA and protein contents are of particular interest as they include miRNAs, shRNA, lncRNAs and components of the immune system.

ExVs become especially important when considering the movement of cell signals from tumor cells to healthy cells when they can help cancer cells to propagate genetic information that leads to the development and maintenance of metastases. Equally, such ExVs may be exploited in the treatment of tumors by acting as carriers of anti-tumor compounds.

2. Exosomal RNAs

ExVs contain varying amounts of RNAs concerning over a dozen different RNA forms [5], the majority of which are classified as ncRNAs (non-coding RNAs). They do not normally code for protein synthesis, but they are able to influence mRNAs in either stabilizing mRNAs, so serving to protect mRNA from degradation, or degrade mRNA. Nevertheless, it is important to know whether or not any mRNA molecules present are intact since they will encode for functional proteins and hence transfer functions of the original cell into the recipient cell. Moreover, partially degraded molecules could deliver new traits into the target cell[13]. Of the ncRNAs, perhaps the two most important in current ExV cancer studies are miRNAs and lncRNAs. Given the possibility of using ExVs as carriers of molecules to attack tumors, e.g., shRNAs, a brief resume of the RNAs present in ExVs is given.

2.1. Natural Occurring RNAs

2.1.1. miRNAs

miRNAs are produced from an initial RNA strand derived from a host gene involving typical splicing, capping and polyadenylating so leading to the development of mature and active 21–23 nucleotide miRNAs[14][15]. The latter integrate with an RNA-induced silencing complex (RISC) that targets the mRNAs to be degraded or inhibited[5]. This makes them useful markers that are present in liquid biopsies and so for the monitoring of the presence, treatment and reappearance of a particular cancer type.

2.1.2. Sponge circRNAs

Sponges may be formed from both linear and circular RNAs (circRNA). CircRNAs are a class of ncRNAs that are formed by 3′-5′ ligation of an RNA molecule. There are three forms of circRNA depending upon the nuclear origin, i.e., whether formed from introns (ciRNA) or exons (ElciRNA) or exon-introns (ecRNA). CircRNAs are important in the regulation of miRNAs by acting as “miRNA sponges” in e.g., the presence of cancer [16][17]. Primarily found in the cytosol, circRNAs—mainly CIRS-7 and SrycircRNA are involved with the latter having 16 binding sites for miR-138 [17]. CDR1as/CiRS-7 is encoded in the genome antisense to the human locus resulting in the name CDR1. This acts as a sponge having over 60 binding sites of miR-7 (CiRS-7—circular RNA sponge for miR-7) [18]. MiR-7 is also pulled down by circHIPK3 in colon cancer cases. Thus, a mechanism exists to reduce the number of e.g., miR-7 in the cell. However, if such RNA molecules are rapidly required in numbers by the cell, they could be released from the sponge. This possibility is based upon CiRS-7 being sliced by miR-671 so leading to a possibility for the existence of a system to release miRNAs at an appropriate time[19]. Zhang et al. [20] have demonstrated that circTRIM33-12 can act as a sponge for miR-19 in hepatocellular carcinoma (HCC). Downregulated circTRIM33-12 was found to upregulate TET1 (ten-eleven translocation methylcytosine dioxygenase 1) expression on sponging miR-191. However, downregulation of circTRIM33-12 in HCC was significantly correlated with malignant characteristics affecting both overall survival and recurrence-free survival after surgery. This basic prognosis appears to be due to the circTRIM33-12 sponging miR-191 and upregulating TET1 expression and hence, leading to significantly reduced 5-hydroxymethylcytosine levels in HCC cells.

Until now, very few circRNAs have been shown to have sponge characteristics[21][22].

2.1.3. lncRNAs

lncRNAs are a class of RNA having more than 200 nucleotides though lacking the potential to code for proteins[19][20]. lncRNAs play significant roles in gene regulation and expression and participate in a variety of biological processes that include imprinting, cell growth, apoptosis and differentiation[19][20]. Their expression levels become dysregulated in patients having different cancer types in association with tumorigenesis, cancer progression and metastases [21].

More than 210,000 lncRNAs are present [22], 106,063 being human related (LncRNAWiki, 2015) though only 14,470 were recorded in Gencode (2014). Of these, only 1867 human lncRNAs appeared to be biologically active in humans [23]. Although there is a very large number of lncRNAs that have been identified, very few have been considered for ExV lncRNA involvement in cancers and cancer resistance to treatment. For example, only HOTAIR, AGAP2-AS1, SNHG14 and UCA1 have been considered for breast cancer resistance to tamoxifen and trastuzumab[24] [25][26][27].

2.2. Artificial miRNAs

shRNAs

shRNAs are artificially created molecules with a tight hairpin turn that gives them the capacity to silence target gene expression via RNA interference (RNAi). They are usually delivered by through plasmid delivery though they are suitable as an exosome component to attack tumor cells. Initially, the cytosolic delivery of RNAi oligonucleotides was only used

with cells capable of transfection and, in particular, transient in vitro studies. With the advent of its use in gene function studies, the need for a more reliable mechanism of delivery was needed. An improved method involved the development of shRNA which permitted infection with viral vectors leading to stable integration of shRNA and long-term knockdown of the targeted gene[28].

shRNAs integrate into the DNA by virtue of two complementary 19–22 bp RNA sequences linked by a short loop of 4–11 nucleotides that resembles the hairpin occurring naturally in miRNAs. After transcription, the shRNA sequence moves to the cytosol where it is processed by the Dicer system as is the case with miRNAs. Subsequently, the siRNA so produced binds to the relevant mRNA and the complex moves to the RISC where the mRNA is broken down[29].

References

1. Zijlstra, A.; Di Vizio, D. Size matters in nanoscale communication. Nat. Cell Biol. 2018, 20, 228–230, doi:10.1038/s41556-018-0049-8.
2. Caby, M.P., Lankar, D.; Vincendeau-Scherrer, C.; Raposo, G.; Bonnerot, C. Exosomal-Like Vesicles Are Present in Human Blood Plasma. Int. Immunol. 2005, 17, 879–887, doi:10.1093/intimm/dxh267.
3. Admyre, C.; Grunewald, J.; Thyberg, J.; Gripenback, S.; Tornling, G.; Eklund, A.; Scheynius, A.; Gabrielsson, S. Exosomes with Major Histocompatibility Complex Class Ii and Co-Stimulatory Molecules Are Present in Human Bal Fluid. Eur. Respir. J. 2003, 22, 578–583, doi:10.1183/09031936.03.00041703.
4. Admyre, C.; Johansson, S.M.; Qazi, K.R.; Filén, J.-J.; Lahesmaa, R.; Norman, M.; Neve, E.P.A.; Scheynius, A.; Gabrielsson, S. Exosomes with Immune Modulatory Features Are Present in Human Breast Milk. J. Immunol. 2007, 179, 1969–1978, doi:10.4049/jimmunol.179.3.1969.
5. Andre, F.; Schartz, N.E.C.; Movassagh, M.; Flament, C.; Pautier, P.; Morice, P.; Pomel, C.; Lhomme, C.; Escudier, B.; Le Chevalier, T.; et al. Malignant effusions and immunogenic tumour-derived exosomes. Lancet 2002, 360, 295–305, doi:10.1016/s0140-6736(02)09552-1.
6. Pisitkun, T.; Shen, R.-F.; Knepper, M.A. Identification and proteomic profiling of exosomes in human urine. Proc. Natl. Acad. Sci. USA 2004, 101, 13368–13373, doi:10.1073/pnas.0403453101.
7. Lässer, C.; O'Neil, S.E.; Shelke, G.V.; Sihlbom, C.; Hansson, S.F.; Gho, Y.S.; Lundbäck, B.; Lötvall, J. Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation. J. Transl. Med. 2016, 14, 181, doi:10.1186/s12967-016-0927-4.
8. Mathieu, M.; L. Martin-Jaular, L.; Lavieu, G.; Thery, C. Specificities of Secretion and Uptake of Exosomes and Other Ex-tra-cellular Vesicles for Cell-to-Cell Communication. Nat. Cell Biol. 2019, 21, 9–17, doi:10.1038/s41556-018-0250-9.
9. Luga, V.; Zhang, L.; Viloria-Petit, A.M.; Ogunjimi, A.A.; Inanlou, M.R.; Chiu, E.; Buchanan, M.; Hosein, A.N.; Basik, M.; Wrana, J.L. Exosomes Mediate Stromal Mobilization of Autocrine Wnt-PCP Signaling in Breast Cancer Cell Migration. Cell 2012, 151, 1542–1556, doi:10.1016/j.cell.2012.11.024.
10. Raposo, G.; Nijman, H.W.; Stoorvogel, W.; Liejendekker, R.; Harding, C.V.; Melief, C.J.; Geuze, H.J. B lymphocytes secrete antigen-presenting vesicles. J. Exp. Med. 1996, 183, 1161–1172, doi:10.1084/jem.183.3.1161.
11. Yakimchuk, K. Exosomes: Isolation and Characterization Methods and Specific Markers. Mater Methods 2015, 5, 1450–1453, doi:10.13070/mm.en.5.1450.
12. Liu, F.; Vermesh, O.; Mani, V.; Ge, T.J.; Madsen, S.J.; Sabour, A.; Hsu, E.-C.; Gowrishankar, G.; Kanada, M.; Jokerst, J.V.; et al. The Exosome Total Isolation Chip. ACS Nano 2017, 11, 10712–10723, doi:10.1021/acsnano.7b04878.
13. Lim, J.; Choi, M.; Lee, H.; Kim, Y.-H.; Han, J.-Y.; Lee, E.; Cho, Y. Direct isolation and characterization of circulating exosomes from biological samples using magnetic nanowires. J. Nanobiotechnology 2019, 17, 1–12, doi:10.1186/s12951-018-0433-3.
14. La Shu, S.; Yang, Y.; Allen, C.L.; Hurley, E.; Tung, K.H.; Minderman, H.; Wu, Y.; Ernstoff, M.S. Purity and yield of melanoma exosomes are dependent on isolation method. J. Extracell. Vesicles 2020, 9, 1692401, doi:10.1080/20013078.2019.1692401.
15. Le Gall, L.; Ouandaogo, Z.G.; Anakor, E.; Connolly, O.; Browne, G.B.; Laine, J.; Duddy, W.; Duguez, S. Optimized method for extraction of exosomes from human primary muscle cells. Skelet. Muscle 2020, 10, 1–13, doi:10.1186/s13395-020-00238-1.
16. McKelvey, K. J.; Powell, K. L.; Ashton, A. W.; Morris, J. M.; McCracken, S. A. Exosomes: Mechanisms of Uptake. J. Circ. Bi-omark. 2015, 4, 7, doi:10.5772/61186.

17. Schwarzenbach, H.; Gahan, P.B. MicroRNA Shuttle from Cell-To-Cell by Exosomes and Its Impact in Cancer. Non-Coding RNA 2019, 5, 28, doi:10.3390/ncrna5010028.
18. Wu, M.; Wang, G.; Hu, W.; Yao, Y.; Yu, X.-F. Emerging roles and therapeutic value of exosomes in cancer metastasis. Mol. Cancer 2019, 18, 1–11, doi:10.1186/s12943-019-0964-8.
19. Kalluri, R.; LeBleu, V.S. Discovery of Double-Stranded Genomic DNA in Circulating Exosomes. Cold Spring Harb. Symp. Quant. Biol. 2016, 81, 275–280, doi:10.1101/sqb.2016.81.030932.
20. Huang, X.; Yuan, T.; Tschannen, M.; Sun, Z.; Jacob, H.J.; Du, M.; Liang, M.; Dittmar, R.; Liu, Y.; Liang, M.; et al. Characteriza-tion of human plasma-derived exosomal RNAs by deep sequencing. BMC Genom. 2013, 14, 1–14, doi:10.1186/1471-2164-14-319.
21. Choi, D.-S.; Kim, D.-K.; Kim, Y.-K.; Gho, Y.S. Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. Pro-teom. 2013, 13, 1554–1571, doi:10.1002/pmic.201200329.
22. Wang, X.; Weidling, I.; Koppel, S.; Menta, B.; Ortiz, J.P.; Kalani, A.; Wilkins, H.M.; Swerdlow, R.H. Detection of mitochon-dria-pertinent components in exosomes. Mitochondrion 2020, 55, 100–110, doi:10.1016/j.mito.2020.09.006.
23. Kim, K.M.; Abdelmohsen, K.; Mustapic, M.; Kapogiannis, D.; Gorospe, M. RNA in extracellular vesicles. Wiley Interdiscip. Rev. RNA 2017, 8, e1413, doi:10.1002/wrna.1413.
24. Lee, Y.; Kim, M.; Han, J.; Yeom, K.-H.; Lee, S.; Baek, S.H.; Kim, V.N. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004, 23, 4051–4060, doi:10.1038/sj.emboj.7600385.
25. Bortolin-Cavaillé, M.-L.; Dance, M.; Weber, M.; Cavaillé, J. C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. Nucleic Acids Res. 2009, 37, 3464–3473, doi:10.1093/nar/gkp205.
26. Zheng, Q.; Bao, C.; Guo, W.; Li, S.; Chen, J.; Chen, B.; Luo, Y.; Lyu, D.; Li, Y.; Shi, G.; et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat. Commun. 2016, 7, 11215, doi:10.1038/ncomms11215.
27. Peng, L.; Chen, G.; Zhu, Z.; Shen, Z.; Du, C.; Zang, R.; Su, Y.; Xie, H.; Li, H.; Xu, X.; et al. Correction: Circular RNA ZNF609 functions as a competitive endogenous RNA to regulate AKT3 expression by sponging miR-150-5p in Hirschsprung's dis-ease. Oncotarget 2019, 10, 3313–3314, doi:10.18632/oncotarget.26963.
28. Hansen, T. B.; Wiklund, E. D.; Bramsen, J. B.; Villadsen, S. B.; Statham, A. L.; Clark, S. J.; Kjems, J. Mirna-Dependent Gene Silencing Involving Ago2-Mediated Cleavage of a Circular Antisense RNA. EMBO J. 2011, 21, 4414–4422, doi:10.1038/emboj.2011.359.
29. Memczak, S.; Jens, M.; Elefsinioti, A.; Torti, F.; Krueger, J.; Rybak, A.; Maier, L.; Mackowiak, S. D.; Gregersen, L. H.; Mun-schauer, M.; et al. Circular RNAs Are a Large Class of Animal RNAs with Regulatory Potency. Nature 2013, 495, 333–338, doi:10.1038/nature11928.