EXOSOMES IN CML DEVELOPMENT AND PROGRESSION

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Abstract: Exosomes are small extracellular vesicles shed by wide variety of cell types. These vesicles can be found in almost any human`s body fluid and serve as carriers of signal molecules and regulating factors involved in many different physiological and pathological processes. The presence of exosomes during Chronic Myeloid Leukemia development and progression as well as their role in modulation of the tumor microenvironment is well studied. Due to the high specificity of the exosomal membrane receptors these vesicles deliver their cargo to the target cells with a precision characteristic for highly evolved biological systems. Investigation of the exosomal formation, release and target association could contribute substantially to the advancements of the modern medicine. Further, exosomes may serve not only as a tool for diagnostics and in disease prognosis but to be implemented as part of the active treatment regimens of the CML patients.

Key words: exosomes, CML, miRNA, metastasis, angiogenesis

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of the early stem cell myeloid progenitors, characterized by the presence of a reciprocal chromosomal translocation t(9:22), which results in synthesis of a fusion BCR-ABL onco-protein (1,2). BCR-ABL is constitutively activated tyrosine kynese which activate a number of downstream signaling cascades (3). BCR-ABL is targeting Ras, PI3K/Akt and Jak/Stat signaling pathways, which regulate cell proliferation and apoptosis. The fusion TK is eliminating leukemia cells dependence on external growth factors by upregulation interleukin-3 production and altering the cell adhesion properties by modulating expression and activation of local adhesion kinase and associated proteins (4,5). The characteristics of the chronic phase of CML, proliferation of the malignang myeloid population and premature release in circulation can therefore be explained by activation of mutagenic pathways, antiapoptotic pathways. These same characteristics, increased mutagenicity and decrease susceptibility to apoptotsis may also be responsible for disease progression (6). Different options of treatment have been explored in CML, including arsenic trioxide, splenic irradiation, hydroxycarbamide, busulphan, but all of them can only control the proliferation of white blood cells with no effect over the disease progression to accelerated phase and blast crisis. The introduction of INF-α induced cytogenetic remission and increased survival (7), especially in patients with early chronic phase (8,9). The only curative treatment is stem cell transplantation, however, as long as the average age of onset is >50 years of age, together with the inability to find suitable donor limits this option for most of the patients (10). The invention of imatinib mesylate, a selective tyrosine-kinase inhibitor, the cytogenetic response and prognosis of CML

chronic phase has significantly improved. Imatinib acts as a competitive inhibitor of ATP, which inhibits the neoplastic clone proliferation and has a significant effect on haematological and cytogenetics level. However, some patients with advanced disease may develop resistance to Imatinib caused by secondary mutations or due to amplification of BCR-ABL (11). Additionally the interaction between the leukemic cells and the bone marrow play an important part in CML pathogenesis (12). In this respect, interesting opportunity is investigating the possibilities to manipulate the microenvironment regulating the growth, survival and drug-resistance of leukemic cells (13). Both, cancerous and non-cancerous cells are exchanging large variety of cytokines, adhesion molecules, growth factors, mediating intercellular communication within the tumor microenvironment, and thus building a suitable niche for leukemia cell growth and expansion. Recently, a lot of information has been gathered regarding the functional role of exosomes in the crosstalk between normal and cancerous cells serving as carriers of various signal molecules through the tumor microenvironment. Exosomes are cell derived vesicles, present in many eukaryotic fluids with diameter between 30 and 100 nm. Exosomes are either released from the cell when multivesicular bodies fuse with the plasma membrane or released directly from the plasma membrane (14). Further, exosomes have been identified to play central role in modulation of interactions between cancer cells and microenvironment (15,16). It is also well documented, that CML cells are shedding large amount of exosomes that interact with endothelial cells as well as with bone marrow stromal cells, leading to progression of the disease (15,17,18).

Angiogenesis and CML

The formation of new blood vessels is defined as angiogenesis. It is complex process of modulation of the extracellular matrix proteins, proliferation and migration of endothelial cells taking place in several steps (20). Various growth factors are implicated in angiogenesis, some of them with stimulating effect [fibroblast growth factor-2 (FGF-2), transforming growth factors (TGF-α, TGF-β), matrix metalloproteinases (MMPs), IL-8], and other with suppressing role [tissue inhibitors of metalloproteinases (TIMPs) and platelet factor-4 (PF-4)], with precise physiologic control over them (21,22). In cancer, the balance between those factors is disrupted due to the release of cytokins by myeloid cells stimulating the endothelial cells of the host to produce new blood vessels (23). Recent findings about CML demonstrate that the disease progression is linked with angiogenesis (24). Evidence for this are the measured higher levels of angiogenic factors like FGF-2 and HGF together with increased vascularization of the marrow (25). BCR-ABL also has a role in CML angiogenesis, which is why a possible interaction between leukemia cells and the bone marrow in the pathogenesis of CML has been hypothesized (19).

Exosome secretion

Exosomes are membrane vesicles produced by the endoplasmic reticulum and have been reported to be secreted by many different cell types like epithelial cells (29), mast cells (27), T-cells (27), B-cells (26), platelets (28) and many more. With the help of electron microscopy (EM), the EV have been observed present in the late endosomes, which are released in the extracellular space through process of merging of endosomes with the cell membrane (30,31). They have been isolated from both, cultured cells and from body fluids like plasma (32), synovial fluid (34), urine (33), and seminal plasma (prostasomes) derived from prostate cells (35). As part of the multivesicular bodies (MVBs), exosomes originate from intraluminal vesicles (ILVs) produced after inward budding of the endosomal membrane, which is why they contain cytosol from the cell. MVBs take part in transportation of proteins, and after merging with plasma membrane they can deliver intraluminal vesicles in the extracellular space, and thus releasing exosomes (31). During formation of the ILVs, the proteins and lipids are packed within membrane of the endosomes, therefore

the secreted exosomes contain cargos corresponding to the environment of the late endosomes (36).

Biochemical composition of exosomes

The exosomes composition strongly depends on the type of cell which they are secreted from and the biological process happening within the endosomes. Using different analytical methods the presence of many common as well as cell-type specific proteins has been reported. For example exosomes from mast cells, B cells and DCs contain molecules like CD54, CD80 and CD86 along with MHC class I and class II complexes (38,39). Exosomes from reticulocytes contain transferrin receptor (41), while cytotoxic T cell derived exosomes contain specific proteins like perforin and granzymes (40). Important part of the exosomes` membrane is the family of the so called tetraspanins (39). Tetraspanins play a critical role in migration, spreading and are important in regulation of integrin compartmentalization, recycling and signaling (42). Tetraspanins also could influence invasiveness by modulating biosynthesis of MMPs as well as regulating cell trafficking and adhesion (43). Typical examples of tetraspanins are CD9, CD63, and CD81. Another important constituent of exosomes are heat shock proteins like Hsp90, Hsp70, Hsc71 (44,37). Heat shock proteins are involved not only in the protein folding processe, but also in antigen presentation by binding to antigen peptides and loading them onto MHC molecules (45). Exosomes include signal transduction molecules, various metabolic enzymes, and carry on their surface CD55 and CC59 molecules allowing them avoid complement lysis (46). Sets of all specifically expressed molecules could be found on the surface of exosomes, such as integrins (αM on DCs, β2 on DCs and T cells), immunoglobulins (A33 antigen on enterocytes, P-selectin on platelets, intercellular adhesion molecule 1 (ICAM1) and CD54 on B cells).

Another very important payload of exosomes are different types of mRNAs and miRNAs. For example the microarray assessment of exosomes, released by human mast cells contain approximately 1300 different RNAs (47). MicroRNAs (miRNAs) are small non-coding nucleotides with average length of 22 nucleotides that possess regulatory functions on gene expression. The life cycle of miRNAs starts with transcription of primary miRNA (pri-miRNA) from the genome by RNA polymerase II. At the next step the nuclear RNase Drosha process pre-miRNAs into hairpin precursor miRNA (pre-miRNA), which are then transported into the cytoplasm via Exportin-5, where a mature miRNA is formed (48). Then, one strand of the mature miRNA is integrated into Argonaute (Ago) protein-containing miRNA-induced silencing complex (miRISC) which consecutively targets mRNA and as result lead to repression of gene expression (49). Additionally, Ago from miRISC complex could interact with Dcp1 and Dcp2 proteins of the so-called P-bodies (50) (cytoplasmic foci, comprised of enzymes involved in RNA turn-over), the resulted formation fuses with late endosomes which in turn releases miRNA-containing exosomes. Exosomes are then taken up by a target cells via receptor-ligand interaction, interfering with recipient cells genes. Interestingly, the latest research in the field have shown that exosomal miRNAs are free of Ago (51) and more than 90% of Ago bounded miRNAs are found outside of extracellular vesicles (52,53). The active miRISCs have been found to be incorporated into the early endosomes (54), which in a later time point is separated from miRNAs that are selectively incorporated by hnRNPA2B1 and hnRNPA1 (55) into late endosomes before exosome release.

Exosomes in cancer

At the very early stages of cancer development cancer cells are starting modifying the environment by affecting intercellular communications and by secretion of different cytokins and factors as TNFα, TGFβ, VEGF, which are responsible for activation of numerous different cell types. Moreover, the latest findings have exposed the role of exosomes in cancer

development and progression serving as carriers of signal molecules that trigger specific reactions in the target cells.

Cancer derived exosomes play a central role in processes like tumor invasion and metastasis. For example the extracellular vesicles are involved in development of the premetastatic niche and setting up the microenvironment at distant sites of the organism by remodelling the extracellular matrix, influencing cell to cell communication and by promoting angiogenesis (56). As integrated part of cell-cell interactions, cell adhesion and migration, CD44 is naturally required together with exosomes and soluble stroma in the process of activation of endothelial cells in the premetastatic organ (57,58). Another interesting feature of EVs is the ability to shuttle inhibitors of apoptosis, secreted as a response of stress conditions, promoting cell survival. Typical example is survavin, an anti-apoptotic protein, which can be taken up from the extracellular media and as a result to interfere with the cancer cells apoptotic mechanisms as well as to stimulate replication and metastasis of the host cell (59). Exosomes from certain tumors are also transporting TGF-β, responsible for differentiation of fibroblasts into myofibroblasts and as a result adjusting the stromal properties in fostering tumor growth, vascularizaion and metastasis (60). Tumor-derived exosomes are also capable of directly suppressing the activation of effector T cells by expressing FasL and TRAIL ligands (61, 62). Another example of the EVs ability to regulate immune system is the inhibition of NKG2D dependent cytotoxicity of NK cells and CD8+T cells by a ligands delivered by exosomes secreted from human breast cancer and mesothelioma cell lines (63,64).

Exosomes are also responsible for the exchange of proteins and genetic materials, important for cell to cell communications (65). The presence of miRNAs in exosomes has long ago been described. These miRNAs can alter significantly the plasticity of cancer cells thus promoting metastasis and angiogenesis. The exosomal RNA can not only affect other cancer cells, but also cells within the tumour microenvironment like endothelial cells, fibroblasts and others acting to abrogate the host immune system and stimulate cancer progression.

Exosomes and CML

The recent studies reveled that exosomes secreted by LAMA84 cells increase IL-8 mRNA in HS5 bone marrow stromal cells (BMSCs), which as a result promotes adhesion of LAMA84 cells to HS6 monolayer (66). LAMA84 derived exosomes also can directly promote survival of the releasing cells by lowering expression of pro-apoptotic genes: BAX, BAD and PUMA, elevating expression of anti-apoptotic genes Bcl-xL, BCL-w and BIRC5. Additionally, the secreted exosomes also led to increase of NF-kB and TGF-β1 levels and activated PI3K/Akt and MAPK/ERK signaling pathways (67). Taverna et all, also reported that exosomes generated from LAMA84 cells induced an increase of ICAM-1 and VCAM-1 cell adhesion molecules and IL-8 expression in human vascular endothelial cells (HUVEC), which was paralleled by a dose-dependent increase of adhesion of CML cells to a HUVEC monolayer. Moreover the treatment with exosomes increased the endothelial cell motility accompanied by a loss of VE-cadherin and β-catenin from the endothelial cell surface (68). Another confirmation of the role of exosomes in the formation of tube-like structures is the evidence for exosomes transferring miR-92 and activating Src signaling pathway in HUVEC cells (69)(70). A characteristic feature of CML is the activation of the Src kinase family, necessary for the BCR-ABL signaling (71). Exosomes derived from K562 cells, another CML established cell line, induces phosphorylation of Src and activates downstream pathways (72). Interestingly, this process could be blocked by Dasatinib. Importantly, miRNA-92a, representative of the miR-17-92 cluster, is found in exosomes release by K562 cells, which correlates with downregulated integrin a5 (target gene of miR-92a) in HUVEC cells. Increased secretion of miRNA-92a stimulates substantially

cell migration and tube formation in HUVECs (73).

CML isolated exosomes, secreted in hypoxic conditions possess stronger ability to induse tube formation in HUVECs compared with exosomes from cells conditioned in normoxia. The latter effect could be explained by higher levels of miR-210 in hypoxic leukemia exosomes, which down-regulates the angiogenesis inhibitor - EFNA3 (74).

Conclusions

Clearly, the unique composition of the exosome`s membrane as well as their cargo (signal proteins and miRNAs) makes them an ideal subject for diagnostic analysis for wide variety of diseases and pathological processes. It is well established that exosomes play a crucial role in the process of tumor metastasis by modulation of the tumor microenvironment, manipulating the target cell`s signal pathways and by switching on and off oncogenic genes. Furthermore, exosomes are largely involved in the process of development of chemotherapeutic resistance and cancer recurrence without the presence of any additional genetic mutations. Very promising research area is the investigation of the interactions between the exosomal miRNAs and target cells genes and control mechanisms. The exchange of miRNA molecules is happening passively and actively between many different cell types in bidirectional manner allowing very large variety of cell to cell communications. Understanding the mechanisms of miRNA uptake would elucidate the downstream effects of exosomes over the target cells. The information obtained by exosome analysis would allow physicians in the near future to be able to apply individualized patient therapy. Additionally the exosomes themselves could be implemented as a tool for cancer therapy using them as vehicles for either cancer drugs or miRNAs with therapeutic effects. In CML particularly, the inhibition of exosomes release on one hand and in the same time implementation of extra cellular vesicules as a target delivery systems may be the answer to the successful therapy with very little recurrence rate.

References:

1. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973; 243:290–3.

2. Sonoyama J, Matsumura I, Ezoe S, Satoh Y, Zhang X, Kataoka Y, et al. Functional cooperation among Ras, STAT5, and phosphatidylinositol 3-kinase is required
for full oncogenic activities of BCR/ABL in K562 cells. J Biol Chem. 2002; 277:8076–82.

3. Sonoyama J, Matsumura I, Ezoe S, Satoh Y, Zhang X, Kataoka Y, Takai E, Mizuk IM, Machii T, Wakao H, Kanakura Y. 2002. Functional cooperation among Ras, STAT5, and phosphatidylinositol 3-kinase is required for full oncogenic activities of BCR/ABL in K562 cells. J Biol Chem 277:8076– 8082.

4. Arlinghaus R, Sun T. 2004. Signal transduction pathways in Bcr-Abl transformed cells. Cancer Treat Res 119:239–270.

5. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF. Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. *Nature* 1987; 328: 342–344.

6. Cortez D, Reuther G, Pendergast AM. The Bcr-Abl tyrosine kinase activates mitogenic signaling pathways and stimulates G1- to-S phase transition in hematopoietic cells. *Oncogene* 1997; 15:2333–2342.

7. Talpaz M, McCredie KB, Mavligit GM, Gutterman JU. Leukocyte interferon induced myeloid cytoreduction in chronic myelogenous leukemia. Blood 1983; 62:689- 92.

8. Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999; 340(17):1330-40.

9. Anonymous. Interferon alfa versus chemotherapy for CML: a meta-analysis of seven randomised trias. JNatl Cancer Inst 1997; 89: 1616-1620.

10. Beelen DW. Schaefer UW. Allogenic stem cell transplants in chronic myeloid leukemia. Ann Hematol. 2002; 81 Suppl 2: S45-6.

11. O'Hare T, Eide CA, Deininger MW Bcr-Abl kinase domain mutations, drug resistance, and 12. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 2007; 328:342–344

13. Seke Etet PF, Vecchio L, Nwabo Kamdje AH. Signaling pathways in chronic myeloid leukemia and leukemic stem cell maintenance: key role of stromal microenvironment. Cell Signal. 2012; 24:1883–8.

14. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int J Mol Sci. 2013; 14:5338–66.

15. Corrado C, Raimondo S, Saieva L, Flugy AM, De Leo G, Alessandro R.Exosome-mediated crosstalk between chronic myelogenous leukemia cells and human bone marrow stromal cells triggers an Interleukin 8-dependent survival of leukemia cells. Cancer Lett. 2014; 348:71–6.

16. Huan J, Hornick NI, Skinner AM, Goloviznina NA, Roberts CT, Kurre P. RNA trafficking by acute myeloid leukemia exosomes. Cancer Res. 2013; 73(2):918–29.

17. Corrado C, Flugy AM, Taverna S, Raimondo S, Guggino G, Karmali R, De Leo G, Alessandro R. Carboxyamidotriazole-orotate inhibits the growth of imatinib-resistant chronic myeloid leukaemia cells and modulates exosomes-stimulated angiogenesis. PLoS One. 2012; 7:e42310.

18. Taverna S, Flugy A, Saieva L, Kohn EC, Santoro A, Meraviglia S, De Leo G, Alessandro R. Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis. Int J Cancer. 2012; 130:2033–43.

19. Diaz-Flores L, Gutierrez R, Varela H. Angiogenesis : an update. Histol Histopathol 1994 ; 9:807

20. Rafii S, Mohle R, Shapiro F, Frey BM, Moore MAS. Regulation of hematopoiesis by microvascular endothelium. Leuk Lymphoma 1997; 27:375–386.

21. Bertolini F, Manusco P, Gobbi A, Pruneri G. The thin redline: angiogenesis in normal andmalignant hematopoiesis. Exp Hematol 2000; 28: 993–1000.

22. Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A. The history of the angiogenic swithc concept. Leukemia 2007; 21:44-52

23. Aguayo, A., Kantarjian, H., Manshouri, T., Gidel, C., Estey, E., Thomas, D.,Koller, C., Estrov, Z., O'Brien, S., Keating, M., Freireich, E., Albitar, M., Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 2000; 96 (6):2240–2245

24. Lundberg LG, Lerner R, Sundelin P, Rogers R, Folkman J, Palmblad J Bone marrow in polycythemia vera, chronic myelocytic leukemia, and myelofibrosis has an increased vascularity. Am J Pathol 2000; 157:15–19

25. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 1987; 328:342–344

26. 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: 1161-72.

27. Raposo, G., Tenza, D., Mecheri, S., Peronet, R., Bonnerot, C., Desaymard, C. Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. Mol Biol Cell 1997; 8: 2631-45.

28. Heijnen, H.F., Schiel, A.E., Fijnheer, R., Geuze, H.J., Sixma, J.J. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood 1999; 94: 3791-9.

29. van Niel, G., Raposo, G., Candalh, C., Boussac, M., Hershberg, R., Cerf-Bensussan, N., Heyman, M. Intestinal epithelial cells secrete exosome-like vesicles. Gastroenterology 2001; 121: 337-49.

30. Harding, C., Heuser, J., Stahl,

P. Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes:demonstration of a pathway for receptor shedding. Eur J Cell Biol 1984; 35: 256-63.

31. Pan, B.T., Teng, K., Wu, C., Adam, M., Johnstone, R.M. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 1985; 101: 942-8.

32. 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: 879-87.

33. Skriner K., Adolph, K., Jungblut, P.R., Burmester, G.R. Association of citrullinated proteins with synovial exosomes. Arthritis Rheum 2006; 54: 3809-14

34. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004; 101: 13368-73.

35. Ronquist G, Brody I. The prostasome: its secretion and function in man. Biochim Biophys Acta 1985; 822: 203-18.

36. Kleijmeer MJ, Escola JM, Uytde Haag FG, Jakobson E, Griffith JM,Osterhaus AD, Stoorvogel W, Melief CJ, Rabouille C, Geuze HJ. Antigen loading of MHC class I molecules in the endocytic tract.Traffic 2001; 2: 124-37.

37. Lamparski H, Metha-Damani A, Yao J, Patel S, Hsu DH, Rueqq C, Le Pecq JB. Production and characterization of clinical grade exosomes derived from dendritic cells. J Immunol Methods 2002; 270: 211–26.

38. Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human Blymphocytes. J Biol Chem 1998; 273:20121–20127.

39. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262: 9412-20.

40. Peters PJ, Borst J, Oorschot V, Fukuda M, Krahenbuhl O, Tschopp J, Slot JW, Geuze HJ. Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. J Exp Med 1991; 173:1099–1109.

41. Berditchevski, F. Complexes of tetraspanins with integrins: more than meets the eye. J. Cell Sci. 2001; 114: 4143–4151

42. Winterwood NE, Varzavand A, Meland MN, Ashman LK, Stipp CS. A critical role for tetraspanin CD151 in α3β1 and α6β4 integrindependent tumor cell functions on laminin-5. Mol. Biol. Cell 2006; 17: 2707–2721

43. Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. Induction of heat shock proteins in B-cell exosomes. J Cell Sci 2005; 118: 3631-8.

44. Thery C, Reqnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castaqnoli P, Raposeo G, Amiqorena S. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat-shock protein hsc73. J. Cell Biol. 1999; 147: 599–610.

45. Srivastava, P. Interaction of heat-shock proteins with peptides and antigen-presenting cells: chaperoning of the innate and adaptive immune responses. Annu. Rev. Immunol. 2002; 20: 395–425.

46. Clayton A, Harris CL, Court J, Mason MD, Morgan BP. Antigen-presenting cell exosomes are protected from complement-mediated lysis by expression of CD55 and CD59. Eur J Immunol 2003; 33: 522-31

47. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee J.J, Lotvall J.O. Exosome-mediated transfer of mrnas and micrornas is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 2007; 9: 654–659.

48. Winter J, Jung S, Keller S, Gregory R.I, Diederichs S. Many roads to maturity: MicroRNA biogenesis pathways and their regulation. Nat. Cell Biol. 2009; 11:228–234.

49. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human risc couples microRNA biogenesis and posttranscriptional gene silencing. Cell 2005; 123:631–640.

50. Liu J, Valencia-Sanchez M A, Hannon GJ, Parker R, MicroRNA-dependent localization of targeted mrnas to mammalian p-bodies. Nat. Cell Biol. 2005; 7:719–723.

51. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of mirna effector complexes and modulate miRNA activity. Nat. Cell Biol. 2009; 11:1143–1149.

52. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF,Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA 2011; 108:5003–5008.

53. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microrna expression in human peripheral blood microvesicles. PLoS ONE 2008; 3:e3694

54. Lee YS, Pressman S, Andress AP, Kim K, White JL, Cassidy JJ, Li X, Lubell K, Lim DH, Cho IS, Nakahara K, Preall JB, Bellare P, Sontheimer EJ, Carhew RW. Silencing by small RNAs is linked to endosomal trafficking. Nat. Cell Biol. 2009; 11: 1150–1156.

55. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, Perez-Hernandez D, Vazquez J, Martin-Cofreces, N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Sanchez-Madrid F. Sumoylated HNRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat. Commun. 2013; 4:2980.

56. Peinado HS, Lavotshkin and D Lyden.The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts Seminarsin Cancer Biology 2011; vol. 21, no. 2, pp. 139–146,

57. Nieuwland R, Van Der Post JAM, Lok Gemma CAR, Kenter G, and Sturk A. Microparticles and exosomesin gynecologic neoplasias Seminars in Thrombosis and Hemostasis 2010; vol. 36, no. 8, pp. 925–929.

58. Stoeck A, Keller S, Riedle S, Sanderson MP, Runz S, Le Naour F, Gutwein P, Ludwiq A, Rubinstein E, Altoveqt P. A role for exosomes in the constitutive and stimulus-induced ectodomain cleavage of L1 and CD44. BiochemicalJournal, 2006; vol. 393, no. 3, pp. 609–618,.

59. Khan S , Aspe JR, Asumen MG, Almaguel F, Odumosu O, Acevedo-Martinez S, De Leon M, Langridge WHR, Wall NR. Extracellular, cell-permeable survivin inhibits apoptosis while promoting proliferative and metastatic potential British Journal of Cancer 2009; vol. 100, no. 7, pp. 1073–1086.

60. Webber J, Steadman R, Mason M D, Tabi Z, Clayton and A. Cancer exosomes trigger fibroblast to myofibroblast differentiation Cancer Research 2010; vol. 70, no. 23, pp. 9621– 9630,

61. Andreola G, Rivoltini L, Castelli C, Huber V, Perego P, Deho P, Squarcina P, Accornero P, Lozupone F, Lugini L, Stringaro A, Molinari A, Arancia G, Gentile M, Parmiani G, Fais S. Induction of lymphocyte apoptosis by tumor cell secretion of FasL bearing microvesicles Journal of ExperimentalMedicine 2002; vol.195, no. 10, pp. 1303–1316.

62. Huber V, Fais S, Iero M, Luqini L, Canese P, Squarcina P, Zaccheddu A, Colone M, Arancia G, Gentile M, Sereqni E, Valenti R, Ballabia G, Belli F, Leo E, Parmiani G, Rivoltini L.Human colorectal cancer cells induce Tcell death through release of proapoptotic microvesicles: role in immune escape. Gastroenterology 2005; vol. 128, no. 7, pp. 1796–1804.

63. Clayton A, Tabi Z. Exosomes and the MICA-NKG2D system in cancer. Blood Cells, Molecules, and Diseases 2005; vol. 34, no. 3, pp. 206–213,

64. Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. Human tumor-derived exosomes downmodulate NKG2D expression Journal of Immunology 2008; vol.180, no. 11, pp. 7249–7258.

65. Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM, and Pegtel DM, Exosomes: fit to deliver small RNA, Communicative & Integrative Biology 2010; vol. 3,

pp. 447–450.

66. Corrado C, Raimondo S, Saieva L, Flugy AM, De Leo G, Alessandro R. Exosome-mediated crosstalk between chronic myelogenous leukemia cells and human bone marrow stromal cells triggers an interleukin 8-dependent survival of leukemia cells.

67. Raimondo S, Saieva L, Corrado C, Fontana S, Flugy A, Rizzo A, De Leo G, Alessandro R. Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism. Cell communication signaling 2015; Feb 03;13:8

68. Simona T, Anna F, Saieva L, Kohn EC, Santoro A, Mereviglia S, De Leo G, Allesandro R. Role of exosomes release by chronic myelogenous leukemia cells in angiogenesis International Journal of Cancer 2012; 130(9):2033-2043.

69. Mineo M, Garfield SH, Taverna S, Flugy A, De Leo G, Alessandro R, Kohn EC. Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a Src-dependent fashion. Angiogenesis 2012; 15(1):33-45.

70. Umezu T, Ohyashiki K, Kuroda M, Ohyashiki JH Leukemia cell to endothelial cell communication via exosomal miRNAs. Oncogene. 2013; 32(22):2747-55

71. Li S Leuk Src-family kinases in the development and therapy of Philadelphia chromosome-positive chronic myeloid leukemia and acute lymphoblastic leukemia. Lymphoma. 2008; 49(1):19-26

72. Mineo M, Garfield SH, Taverna S, Flugy A, De Leo G, Alessandro R, Kohn EC Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a Src-dependent fashion. Angiogenesis 2012; 15(1):33-45

73. Umezu T, Ohyashiki K, Kuroda M, Ohyashiki JH Leukemia cell to endothelial cell communication via exosomal miRNAs. Oncogene. 2013; 32(22):2747-55

74. Tadokoro H, Umezu T, Ohyashiki K, Hirano T, Ohyashiki JH Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. J Biol Chem. 2013; 288(48):34343-51

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