

Crosstalk between CML cells with HUVECs and BMSCs through exosomes

Zafer Cetin^{1,2}, Eyup I. Saygili^{3,4} Mehmet Yilmaz⁵

¹Department of Medical Biology, School of Medicine, Sanko University, Gaziantep, Turkey

²Department of Biological and Biomedical Sciences, Graduate Education Institute, Sanko University, Gaziantep, Turkey, ³Department of Medical Biochemistry School of Medicine, Sanko University, Gaziantep, Turkey, ⁴Department of Molecular Medicine, Graduate Education Institute, Sanko University, Gaziantep, Turkey, ⁵Department of Internal Medicine and Hematology, Sanko University Sani Konukoglu Application and Research Hospital, Gaziantep, Turkey

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Signaling Pathways activated in CML
 - 3.1. JAK/STAT pathway
 - 3.2. Ras/Raf/MAPK pathway
 - 3.3. PI3K/Akt/mTOR pathway
 - 3.4. Hedgehog pathway
 - 3.5. Wnt/beta-Catenin pathway
 - 3.6. TGF-beta/TGFB1R pathway
 - 3.7. Notch pathway
4. Tyrosin kinase inhibitors (TKI) in CML
5. Exosomes
6. Exosomal Crosstalk among CML, endothelial and BMSCs
 - 6.1. Selective miRNA expression in CML derived exosomes
 - 6.2. Effects of exosomes on autocrine cell growth in CML
 - 6.3. Crosstalk Between CML And Endothelial Cells Through Exosomes
 - 6.3.1. Exosomal IL8 Mediated Crosstalk
 - 6.3.2. Exosomal miR-210 mediated crosstalk
 - 6.3.3. Exosomal miR-17-92 cluster mediated crosstalk
 - 6.3.4. Exosomal miR-126 mediated crosstalk
 - 6.3.5. Exosomal miR-21 mediated crosstalk
 - 6.4. Crosstalk between CML cells and BMSCs through exosomes
 - 6.4.1. IL8-CXCR1/CXCR2 mediated crosstalk
 - 6.4.2. Exosomal AREG mediated crosstalk
 - 6.4.3. Exosome mediated crosstalk through undefined mechanism
 - 6.4.4. Exosomal miR-711 mediated crosstalk
 - 6.4.5. Exosomal miR-320 mediated crosstalk
 - 6.4.6. Exosomal miR-92a-3p mediated crosstalk
7. Conclusion remarks
8. Acknowledgments
9. References

1. ABSTRACT

Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm characterized by presence of the *BCR-ABL* fusion gene which encodes the constitutively active BCR-ABL chimeric protein. Imatinib is first FDA approved first-line BCR-ABL targeting drug for the treatment of newly diagnosed CML cases. Nowadays there are recently developed and more efficient TKIs in the market. Despite the improvements in the CML therapy by using tyrosine kinase inhibitors (TKIs) primary/secondary resistance or progression from chronic to accelerated and blastic phase may be developed in some cases. Underlying mechanisms of TKI resistance and disease progression may results from BCR/ABL dependent or independent alterations. Recently it was revealed that tumor microenvironment is very important for cancer cell growth, survival, proliferation, hemostasis, invasion and metastasis. Exosomes derived from tumor cells contain many important signaling molecules and transfer these molecules in the neighbouring cells. In the bone marrow matrix CML cells, CML leukemic stem cells, cells, bone marrow mesenchymal stromal cells can communicate with each other through exosomes. In this review we focused on biological and clinical importance of CML derived exosomes and we will summarize the recent studies in this field.

2. INTRODUCTION

CML is a myeloproliferative disorder, characterized by the expansion of pluripotent bone marrow stem cells. The hallmark of the disease is presence of a small derivative chromosome 22 known as the Philadelphia chromosome resulting from a classical balanced reciprocal translocation t(9;22)(q34;q11.2) in leukemia cells. Approximately in 90-95% of the newly diagnosed CML cases balanced t(9;22)(q34;q11.2) translocation could be detected during cytogenetic analysis from bone marrow sampling (1). Variant Philadelphia translocations resulting from translocation between three or more chromosomes could be observed in the remaining 5-10% of the newly diagnosed chronic phase CML cases (2-3). Three different clinical stages are defined for CML including; the chronic phase (CML-CP), the accelerated phase (CML-AP), and the blast

crisis (CML-BC). During the progression of the disease through CML-CP to CML-AP and CML-BC increased genomic instability and gradually increased number of structural and numerical chromosomal abnormalities could be detected in CML cells (4).

Translocation of the proto-oncogenic tyrosine-protein kinase *ABL1* gene located on chromosome 9 to the breakpoint cluster region (*BCR*) gene located on chromosome 22 results in generation of a *BCR-ABL1* fusion gene on the Philadelphia chromosome in CML cells. Chimeric BCR-ABL1 fusion protein which encoded by *BCR-ABL1* fusion gene have constitutive tyrosine kinase activity. Depending on the breakpoints in the *BCR* and *ABL1* genes there are three types of BCR-ABL fusion transcripts including; Major BCR-ABL1 (p210), minor BCR-ABL1 (p190) and micr BCR-ABL1 (p230). In the majority of CML cases only major BCR-ABL1 transcript has been expressed (5).

3. SIGNALING PATHWAYS ACTIVATED IN CML

In the presence of BCR/ABL1 fusion protein ABL1 oncoprotein have constitutive activity and phosphorylate a broad range of signaling proteins which activates different intracellular signaling pathways. These signaling pathways include; JAK/STAT, RAS/RAF/MAPK, PI3K/Akt/mTOR, Hedgehog (Hh), Wnt/beta-Catenin, TGF-beta/TGFBR, Notch signaling pathways involving in cell survival, proliferation, inhibition of apoptosis, alteration in cell adhesion properties, angiogenesis, extracellular matrices remodeling, stemness of leukemia stem cells (6).

3.1. JAK/STAT pathway

JAK/STAT signaling is one of the important pathway which is activated in CML. When a growth factor or a cytokine binds to its receptor it results in; formation of receptor chain aggregate, accumulation of Janus Kinase (JAK1-3 and Tyk2) into close proximity, transphosphorylation of the JAKs at tyrosine residues, cytokine receptor chain

phosphorylation, binding of Signal Transducer and Activator of Transcription-proteins (STAT1-4, STAT5A, STAT5B, and STAT6) to phosphotyrosine residues of the receptor tyrosine kinase, tyrosine phosphorylation of STATs through JAKs followed by dissociation of STATs from the receptor tyrosine kinase complex, STAT dimerization through phosphotyrosine-SH2 interactions followed by translocation to the nucleus where they bind to regulatory regions of target genes containing STAT-binding motifs (*Cyclin D1*, *Bcl-X*, *Mcl1*, *MDR1*, *TERT*, etc.) (7-9).

3.2. Ras/Raf/MAPK pathway

Second signaling pathway which is activated through BCR/ABL1 is Ras/Raf/MAPK pathway. Binding of a growth factor to its receptor results in; receptor dimerization, tyrosine transphosphorylation of the receptor chains, binding of Growth Factor Receptor Bound protein 2 (Grb2) to phosphotyrosine residues, interaction between Grb2 and Son of Sevenless (SOS), Ras-GDP recruitment to the cell membrane, formation of active Ras-GTP complex, activation of serine/threonine protein kinase Raf-1, activation of MEK, activation of serine/threonine protein kinase MAPKs. There are three branches of the MAPK cascade including; Extracellular signal Regulated Kinase (ERK), c-Jun N-terminal Kinase (JNK), and p38 MAP kinase. Activated ERK translocate into the nucleus where they phosphorylate the transcription factors (c-MYC, Ets, c-Fos, Elk, c-Jun) as substrates and regulate target genes transcription (10).

3.3. PI3K/Akt/mTOR pathway

Phosphatidylinositol-3-Kinase (PI3K) is another target of the BCR-ABL. Activated PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), phosphatase and tensin homologue (PTEN) downregulates signaling cascades induced by PI3K, PIP3 activates phosphoinositide-dependent kinase 1 (PDK1) and Akt (11). Phospho-Akt block both Tuberous Sclerosis 1 and 2 (TSC1 and TSC2) complexes, GTP bound Ras Homolog Enriched in Brain (Rheb) increase and activates its downstream proteins including Mammalian Target of Rapamycin

(mTOR) which are the catalytic subunit of two protein complexes: mTORC1 and mTORC2 (12). These two complexes present different compositions and functions. mTORC1, composed by mTOR, Regulatory Associated Protein of mTOR (RAPTOR), mLST6 and PRAS40 activates S6 Kinase (S6K), S6 ribosomal protein (rpS6) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) controlling cell cycle (progression from G₁ to S phase), cell proliferation, survival, and angiogenesis. The mTORC2 is composed by mTOR, Rapamycin-Insensitive Companion of mTOR (RICTOR), mLST6, Protein observed with Rictor1 (Protor), and SAPK Interacting protein 1 (mSIN1), regulate cytoskeleton organisation and cell proliferation (13).

3.4. Hedgehog pathway

One of the major pathways influencing CML leukemia stem cells self-renewal potential is the hedgehog (Hh) signaling pathway. Recent studies show that Hedgehog signaling is upregulated in BCR-ABL⁺ CML progenitor cells and gradually upregulated through the disease progression. There are three isoforms of Hh produced by stromal cells including; Sonic hedgehog (Shh), Desert hedgehog, (Dhh) and Indian Hedgehog (Ihh) (14). Hh proteins secrete by the Dispatched (Disp) into extracellular media. In the absence of Hh, Patched1 (Ptc) inhibits the activity of the Smoothened (Smo). In the receiver cell Hh binds to its receptor Ptc. Binding of Hh to Ptc results in loss of Ptc activity followed by activation of Smo leading to the activation of the glioma-associated oncogene family (GLI1-3) of transcription factors. GLI transcription factors dissociate from Suppressor of Fused (SUFU) and translocate to the nucleus where they regulate the expression of target genes associated with cell cycle, proliferation and survival (*CYCLIND*, *c-MYC*, *BCL-2* etc.) (15)

3.5. Wnt/beta-Catenin pathway

Wnt signaling also important in CML cell stemness. In the canonical pathway when Wnt signaling is not activated, beta-Catenin marked for degradation by a multiprotein complex that is composed of Axis Inhibitor (Axin), Adenomatous Polyposis Coli (APC), Glycogen Synthase Kinase 3 (GSK-3), Creatin Kinase 1 (CK1), protein

phosphatase 2A (PP2A) and E3-ubiquitin ligase (beta-TrCP) followed by degradation by proteasomes (16). Binding of the Wnt to the its Frizzled (Fzd) receptor and to the co-receptor Low-density lipoprotein Receptor-related Protein (LRP5/6) results; phosphorylation of Dishevelled (Dvl), formation of LRP aggregates, phosphorylation of LRP receptors, inhibition of degradation complex, and translocation of beta-Catenin into the nucleus (17). Nuclear beta-Catenin interacts with members of the Tcf/Lef transcription factors (Tcf1, Tcf3-4 and Lef1) and binds to co-activator proteins; PRO paired like homeobox-1 (Prop1), forkhead box O (FOXO), Krueppel-like factor 4 (Klf4) activating the target gene expression including (*c-MYC* and *CYCLIND1*, etc.) (18).

3.6. TGF-beta/TGFB1R pathway

Constitutive BCR/ABL activity upregulate the expression of Transforming Growth Factor, Beta-1 (TGF-beta 1) and quiescent CML stem cells express higher levels of TGF-beta 1. TGF-beta binds to TGF-BRII and TGF-BRI dimers which have serine-threonine kinase activity. TGF-BRII homodimer phosphorylates TGF-beta RI homodimers (19). Activated TGF-beta receptor complex recruits Smad and Mad-Related Protein 2-3 (Smad2-3) and phosphorylates them. Phosphorylated Smad 2 and 3 form a trimeric complex with Smad4 which translocate to the nucleus and regulate target gene expression. In the route of non-canonical TGF-beta pathway Erk, JNK and p38 MAP kinase and PI3K-Akt pathways are also activated. Activation of PI3K-Akt-NF-kB pathway results in upregulation of Matrix Metalloproteinase 9 (MMP9), which further stimulates the expression of Kit Ligand (sKITL) and Intercellular Adhesion Molecule-1 (sICAM-1) (20). sICAM-1 prevents the CML cells from the recognition by T lymphocytes and natural killer cells while s-KITL leads to mobilization of CML cells to the peripheral circulation. TGF-beta-Akt signalling also induce the FoxO3a pathway, leading to CML-initiating cells maintenance (21).

3.7. Notch pathway

Another signaling pathway important in proliferation, cell survival, and hematopoietic

stem-cell self-renewal is Notch pathway. Notch receptors (Notch1-4) have been activated through interaction with their ligands including; Serrate-like Jagged1-2 (JAG1-2) and Ligand of the Delta-like (Delta1, 3-4) (22). Notch extracellular domain (NECD) present on the membrane of signal-receiving cells binds Notch ligands expressed on the membrane of signal-sending cells. This receptor-ligand interaction mediates two sequential proteolytic cleavages in the Notch through A-Disintegrin and Metalloprotease-10 and 17 (ADAM-10, ADAM-17) and Presenilin (PS)/ γ -secretase complex (23). After these cleavages Notch intracellular domain (NICD) translocate to the nucleus. In the nucleus NICD associates with Recombination Signal-Binding Protein for Immunoglobulin Kappa J Region (RBPJ) and Mastermind-like1-3 (MAML1-3) resulting in activation of target genes transcription including; *Hairy/Enhancer of Split 1/5 (HES1/5)*, *Hairy/Enhancer of Split-Related with YRPW Motif (HEY)*, *Myc*, *cyclinD*, *Pre-T-Cell Receptor*, *Alpha-Chain Precursor (pTalpha)*, and *JAG1* (24).

4. TYROSIN KINASE INHIBITORS (TKI) IN CML

Detection of importance of BCR/ABL oncoprotein in CML led to improvements in researches on drug design and development targeting this oncogenic protein. Imatinib mesylate was the first TKI to receive the Food and Drug Administration (FDA) approval for the treatment of patients with CML-CP. Imatinib binds to the BCR-ABL1 tyrosine kinase domain, change the conformation of ATP binding site and prevent the phosphorylation of the substrate proteins (25). In newly diagnosed CML cases having first-line imatinib mesylate therapy approximately 20% of the cases discontinues the therapy due to intolerance to the drug and approximately another 20% of the cases develops drug resistance during the therapy. First imatinib resistance mechanism was acquired point mutations in the kinase domain of *BCR/ABL* gene during the disease progression. (26). This observations led to the development of second and third generation tyrosine kinase inhibitors that targeting a large spectrum of tyrosine kinase proteins in addition to BCR-ABL including; Nilotinib, Dasatinib,

Bosutinib, (Second Generation TKI), Radotinib, and Ponatinib (Third Generation TKI) (27).

Among the second generation TKIs, Nilotinib inhibits the kinase activity of *BCR-ABL* mutant cell lines except those carrying the T315I mutation and also inhibits Platelet-Derived Growth Factor Receptor (PDGFR- α and PDGFR- β) and c-Kit (28). Dasatinib is a more potent inhibitor of BCR/ABL compared with Imatinib and Nilotinib which inhibit the kinase activity of wild-type and BCR-ABL mutant cell lines except those carrying the T315I mutation. Dasatinib also inhibits Src family of kinases (Fyn, Yes, Src, and Lck), Ephrin Receptor A2 (EphA2), PDGFR, and c-Kit in addition to BCR-ABL (29). Third second-line TKI for CML is Bosutinib. Bosutinib is a dual Src and ABL TKI which inhibits kinase activity of wild-type and *BCR-ABL* mutant cell lines except those carrying T315I and V299L mutations (30).

Among the third generation TKIs, Radotinib inhibits wild-type and mutant *BCR-ABL1* with the exception of T315I. In addition to BCR/ABL Radotinib targets c-Kit, PDGFR and SRC. This drug is currently approved in only South Korea for treatment of newly diagnosed CML cases and insensitive to other BCR/ABL TKIs (31). Another third generation TKI, Ponatinib inhibits wild-type and BCR-ABL mutant cell lines including those carrying the T315I mutation. Ponatinib also inhibits Vascular Endothelial Growth Factor Receptor (VEGFR), PDGFR, Epidermal Growth Factor receptor (EGFR), SRC, KIT, Rearranged During Transfection (RET), FMS-Related Tyrosine Kinase 3 (FLT3) in addition to BCR-ABL (32).

Despite of the improvements in drug development and treatment strategies, in some of the cases resistance to TKIs developed through ABL independent mechanisms including; activation of alternative pathways, gain of non-random chromosomal abnormalities, mutations in the genes associated with DNA repair mechanism, increased activity of multidrug-resistance proteins, variations in cytochrome P450 enzymes, quiescent CML stem cells, epigenetic and microenvironmental alterations regulated by mesenchymal stromal cells, the immune cell contexture in the bone marrow microenvironment (33-34).

Recently it has been demonstrated that exosomes released from CML cells play important roles in cell-cell communication, drug resistance, quiescent CML cell stemness, modulation of cell adhesion, extracellular niche reorganisation, neovascularization and drug resistance. According to recent reports cell to cell communication among CML cells and Endothelial Cells (EC) and Bone Marrow Mesenchymal Stromal Cells (BMSCs) may be involved in drug unresponsiveness or resistance to the drugs, cell surveillance and stemness of CML cells.

5. EXOSOMES

Exosomes are microvesicles (30–150 nm) produced by endocytic network and released from all cell types under physiological as well as pathological conditions into extracellular space. Inward budding of endosomal membranes results in the accumulation of intraluminal vesicles (ILVs) within the large multivesicular bodies (35). Endosomal Sorting Complexes Required for Transport (ESCRT) complexes play important roles in the formation of MVBs. During this process, some cytosolic components including; proteins, lipids and RNAs are sorted into ILVs (36). In addition to ESCRT dependent pathway, exosome biogenesis is also regulated through an ESCRT independent pathway. In this pathway conversion of sphingomyelin to ceramide by Sphingomyelinase (nSMase) play an important role in the ILVs formation. (37). After the fusion of MVB membranes and plasma membranes, content of the ILVs secreted from cells as exosomes. They could be isolated from many body fluids including plasma, urine, semen, saliva, bronchial fluid, cerebral spinal fluid, breast milk, serum, amniotic fluid, synovial fluid, tears, lymph, bile, and gastric acid. There are several exosome isolation methods based on ultracentrifugation, precipitation, targeted filtration, affinity using magnetic beads, size exclusion chromatography which have advantages or disadvantages over each other (38).

Due to exosome formation and release require ESCRT pathway, proteins of this pathway and its accessory proteins including Alix, Tumor Susceptibility Gene 101 (TSG101), Heat-Shock Cognate Protein 70 (HSC70), and Heat Shock

Protein 90 (HSP90) have been found in exosomes regardless of the secreting cell type. In some cells, release of exosomes into the extracellular space depends on sphingomyelinase enzyme activity. In the exosomes released through this pathway tetraspanin family transmembrane proteins including CD9, CD63 and CD81 are enriched in exosomes. Therefore, this set of proteins are called as “exosomal marker proteins.” And different combinations of these proteins have been used in exosome characterization (39).

Physical characterization of the exosomes could be done by using different techniques including; Nanoparticle Tracking Analysis (NTA), Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) and Tunable Resistive Pulse Sensing (TRPS). NTA, DLS and TRPS techniques allows to estimate the particle size and concentration. In addition, exosome concentration could be estimated by using colorimetric quantitation of Acetylcholinesterase activity in the exosomes. TEM and SEM have been used for evaluation of exosomal vesicles morphology (40).

Exosomes contain variety of molecules such as receptors, transcription factors, enzymes, extracellular matrix proteins, microRNAs, long-noncoding RNAs, mRNAs, DNA fragments, lipids and some other metabolites, and their content is affected by different environmental factors and health status. After the secretion of exosomes from secreting cells they can fuse with recipient cells and affect the intracellular signaling pathways and metabolism (41).

Recently, it has been documented that; exosomes play important roles in intercellular signal transduction, cell proliferation, cancer progression, regulation of tumor microenvironment, drug resistance, invasion, metastasis, immunosuppression, angiogenesis in various types of the cancer especially in solid tumors (42-43). There are, published reports on importance of exosomes in CML cell viability, survival and stemness based on cell-cell communication among CML cells, endothelial cells and BMSCs. In this review, we focused on biological and clinical importance of CML cell derived exosomes and exosomal factors.

6. EXOSOMAL CROSSTALK AMONG CML, ENDOTHELIAL and BMSCs

6.1. Selective miRNA expression in CML derived exosomes

Feng *et al.*, (2013) compared miRNA profile of the parental K562 cells and the exosomes of them by microarray analysis. They observed that miRNA expression profile of K562 cells and their exosomes were vary similar. Interestingly, 49 miRNAs expression levels were found to be up-regulated in exosomes compared to K562 cell extracts. Bioinformatical analysis demonstrated that among these 49 up-regulated miRNAs, 25 is associated with the cell cycle, antiapoptosis, cell adhesion, Wnt signaling, angiogenesis, tumor invasion and metastasis, mesenchymal cell proliferation, and cell migration. Among the miRNAs upregulated in exosomes, twenty one were modulators of the Wnt signaling pathway. They also reported that, adherens junction and focal adhesion pathways were also important which were modulated by 19 different miRNAs enriched in K562 derived exosomes (44). Their results suggest that miRNA profiles of parental CML cells and exosomes derived from them might be different. This diversity might be regulated by intracellular mechanisms and provides the advantage to CML cells survival and proliferation.

6.2. Effects of exosomes on autocrine cell growth in CML

It's reported by Raimondo *et al.*, 2015, expression of TGF-beta 1 is higher in exosomes compared with LAMA84 cells that they have TGF-beta 1 receptors at their plasma membrane. Treatment of LAMA84 cells with LAMA84 derived exosomes results in; an increase in the colony formation and proliferation rate, elevation of phospho-SMAD 2/3, elevation of phospho-Akt, phospho-ERK and total Nuclear Factor-kappa B (NF-kB) protein levels. Treatment of LAMA84 cells with LAMA84-derived exosomes leads to reduction of the pro-apoptotic BCL2-Associated Agonist of Cell Death (BAD), BCL2-Associated X (BAX), p53-Upregulated Modulator of Apoptosis (PUMA) proteins expressions and an elevation in the anti-apoptotic BCL2-Related Protein, Long Isoform (BCL-xl), BCL2-Like 2

(BCL2L2) and survivin proteins expressions. Co-treatment of LAMA84 cells with exosomes and TGF-beta 1 receptor inhibitor (SB) or anti-TGF-beta 1 antibody reverses all of these findings. It is also observed in Non Obese Diabetic/ Severe Combine Immunodeficient (NOD/SCID) mice model after 50 days exosome-treated mice develops larger tumors compared with exosome untreated mice. Phospho-ERK, phospho-Akt and total NF-kB protein levels in tumor biopsies obtained from mice treated with exosomes found to be elevated (45). Their results show that, LAMA84 derived exosomes leads to cell survival and proliferation in an autocrine fashion through TGFB1/TGFB1R and activation of both canonical and non-canonical (ERK, PI3K/Akt and NF-kB) pathways *in vitro* and *in vivo* models. Also, LAMA84 derived exosomes can activate antiapoptotic molecules while repress pro-apoptotic molecules.

6.3. Crosstalk between CML and endothelial cells through exosomes

6.3.1. Exosomal IL8 mediated crosstalk

Treatment of HUVECs with 50 µg/ml of LAMA84R-derived exosomes or 10 ng/ml recombinant IL8 increase in phospho-Erk $\frac{1}{2}$, phospho-Akt, Vascular Cell Adhesion Molecule 1 (VCAM1), ICAM1 and IL8 expression in HUVECs leading to increased adhesion of LAMA84R cells to Human Umbilical Vein Endothelial Cells (HUVECs), tube formation and the length of cellular projections. Incubation of exosome induced HUVECs with IL8 siRNA, neutralizing IL8 antibody or Carboxyamidotriazole orotate (CTO) reverses of the affects of exosomes on HUVECs (46).

Protein profiling analysis revealed that contents of LAMA84 cell lines and of exosomes are different. Incubation of HUVECs with LAMA84-derived exosomes, exosomes isolated from CML patients or recombinant IL8 leads to dose- and time-dependent increase in phospho-MAPK p42/44 level, VCAM-1, ICAM-1 and IL8 expressions in HUVECs and LAMA84 cell, and also increase in adhesion to HUVEC monolayer, endothelial cell migration, formation of tubular connections. Incubation of HUVECs with LAMA84-exosomes results in decreased cadherin expression with granular

cytoplasmic localization and translocation of beta-catenin from the plasma membrane to the nucleus. However, incubation of HUVECs with IL8 neutralizing antibody reverses all effects of IL8 (47).

Incubation of HUVECs with K562 exosomes, exosome lysates or recombinant IL8 increase tube formation and angiogenesis in a dose-dependent manner. PKH26-labeled exosomes have been internalized by HUVECs and accumulated in the perinuclear region of the cell during early tubular organization. In the cells reorganizing and sprouting extensions, exosomes move towards the cell periphery and into pseudopods. Within tubular network, exosomes might translocate from cell to cell. In HUVECs treated with K562 exosomes ^{416}Y -Src level and Phospho-Src localization level at membrane interaction regions have been found to be increased (48).

6.3.2. Exosomal miR-210 mediated crosstalk

Incubation of K562 cells under hypoxic conditions for 48–72 h results in elevated expression of Hypoxia Inducible Factor 1 Alpha (HIF-1 alpha). Under hypoxic conditions miR-18b and miR-210 have been overexpressed in both K562 cells and their exosomes. K562 $^{1\%O_2}$ -exosomes containing miR-210 increase the tube and capillary-like structure formation from HUVECs. Exosomal miR-210 directly binds 3' UTR region of the Ephrin A3 (EFNA3) mRNA (anti-angiogenic factor) and degrade it in HUVECs. Treatment of HUVECs with K562 $^{1\%O_2}$ -exosomes under normoxic conditions suppress EFNA3 expression. Transduction of K562 cells with miR-210 inhibitor cultured under hypoxic conditions led to a decrease in endothelial tube formation by HUVECs (49).

Tissue Inhibitor of Metalloproteinase 1 (TIMP-1) overexpression results in upregulation of miR-210 in lung adenocarcinoma cell line A549 through CD63/PI3K/Akt/HIF-1 pathway, accumulation of miR-210 in exosomes, and increased tube formation and angiogenesis in HUVECs, promoted by miR-210 including exosomes (50). It is also reported that, under the hypoxic conditions HIF-1 alpha up-regulate the miR-210 expression in response to the low oxygene condition in tumor cells

and in their exosomes breast cancer cell lines MCF7, MDA-MB 231 and SKBR3 and human lung adenocarcinoma cell line A549 (51-52). Exosomes isolated from the sera of Hepatocellular Carcinoma (HCC) patients and from QGY-7703, HepG2, SK-Hep-1, and Huh-7 cell lines have increased miR-210 level and transfection of HUVECs with miR-210 induce HUVECs to form capillary-like structures and tubulogenesis (53). nSMase2 expression, exosome secretion and miR-210 expression in exosomes is higher in breast cancer cells lines (4T1 cells, MDA-MB-231-D3H1 and MDA-MB-231-D3H2LN) than in normal mammary epithelial cell line (MCF10A). Exosomes and miR-210 transferred from breast cancer cell lines to HUVECs increase the tube formation of HUVECs and metastatic potential of the cancer cells while decrease EFNA3 expression. nSMase2 knocked down and nSMase2 inhibitor (GW4869) reduce transfer of miRNA-210 including exosomes from 4T1- and MDA-MB-231 cells to endothelial cells lung metastatic potential with lower endothelial cell density (54). miR-210-mediated down-regulation of EFNA3 in HUVECs results in increased expression of VEGFR2 which promotes increased VEGF expression and leads to angiogenesis and tubular structure formation from endothelial cells (55). In HCC patients, high miR-210 expression was significantly correlated with poor prognosis and positively correlated with microvascular density (56). miR-210 overexpression also inhibits the expression of sprouty-related protein-1 (SPRED1) and Phosphatidylinositol-3-Kinase Regulatory Subunit 2 PIK3R2 in HUVECs (57).

According to literature we propose cell to cell communication between leukemia cells and HUVECs through miR-210 including: hypoxia induced HIF-1 alpha expression through CD63/PI3K/Akt/HIF-1 pathway, upregulated nSMase2 expression, HIF-1 alpha induced miRNA-210 upregulation and exosomal secretion, internalization of exosomes from HUVECs, increase in phospho-Erk, -Akt, -Src and -Focal Adhesion Kinase (FAK), downregulation of cytoplasm EFNA3, SPRED1 and PIK3R2, increased expression of VEGFR, VEGF, ICAM and VCAM, mislocalisation of e-Cadherin into cytoplasm translocation of Catenin into the nucleus, expression of IL8. This signaling

cascade results in increased vascularisation and angiogenesis in the extracellular niche.

6.3.3. Exosomal miR-17-92 cluster mediated crosstalk

The members of miR-17-92 gene cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b, miR-92a) are overexpressed in K562 cell line. miR-92a packaged in K562 exosomes have been transferred from K562 cells to HUVECs *in vitro* co-culture system. However, incubation of miR-92a overexpressing K562 cells with nSMase inhibitor (GW4869), miR-92a expression level decrease in HUVECs. miR-92a binds 3'-untranslated region of the Integrin $\alpha 5$ (ITGA5) mRNA and decrease the expression of it. Treatment of HUVECs with K562 exosomes overexpressing miR-92a increase the number of migrated HUVECs Also, HUVECs cultured on matrigel treated with K562 exosomes overexpressing miR-92a predispose to form the tube-like structures (58).

miR-17-92 cluster genes upregulated in multiple tumor types (lung, breast, stomach, prostate, colon and pancreatic cancer) but their role in angiogenesis is still controversial. Overexpression of miR-92a in blood samples of colorectal cancer patients associated with invasiveness. And also, expression level of miR-92a were increased in colon cancer cell lines DLD-1, COLO201, SW480, and WiDr. Dickkopf 3 (Dkk-3) is a potential target of miR-92a in colon cancer and Dkk-3 is downregulated in colon cancer cell lines, miR-92a including MVs transferred into the HUVECs through exosomes increase motility and tube formation ability of HUVECs (59). miR-92a-3p levels in the exosomes were higher than those within in colon cancer cell lines DLD-1, COLO201, SW480, and WiDr. Claudin 11 (*CLDN11*) which encodes integral components of tight junctions another target of miR-92a-3p. miR-92a-3p mediated downregulation of CLDN11 promote proliferation, migration, and tube formation in HUVECs. These results indicate that CLDN11 downregulation by exosomes containing miR-92a-3p increase the angiogenesis in endothelial cells (60). Treatment of HUVECs with VEGF, increase the expression of miR-17-92 cluster genes through VEGF/ERK/ELK-1 pathway, proliferation and angiogenic sprouting of endothelial cells, decrease

expression of PTEN and Thrombospondin 1 (THBS1- antiangiogenetic factor) in HUVECs (61). Under the prolonged and cyclic hypoxia conditions HUVECs display a distinctive changes in the miRNA expression profile, among those miRNAs miR-19a-5p expression level was decreased during cyclic hypoxia. HIF-1 alpha, Cyclooxygenase-2 (COX-2), Vascular Endothelial Growth Factor (VEGF), VEGFR1, Endothelin 1 (EDN1), and Endothelin-B Receptor (EDNRB) are potential targets of this miRNA, decreased expression of miR-19a-5p results in elevated EDNRB and increased tube formation by HUVECs (62).

These finding show that, effect of the members of miR-17-92 gene cluster on different cellular processes might be different depending on the cell type, physiological conditions and the external factors that the cells expose and miR-17-92 cluster genes induce angiogenesis through upregulation of HIF-1 alpha, COX-2, VEGF, VEGFR1, EDN1, EDNRB, and downregulation of PTEN and CLDN11.

6.3.4. Exosomal miR-126 mediated crosstalk

It is reported that, miRNA-126 level is higher in LAMA84 exosomes than in the parental LAMA84 cells. Incubation of HUVECs with 20–50 µg/ml LAMA84 exosomes resulted up-regulation in miR-126 expression in HUVECs in a dose-dependent manner. miR-126 binds to 3'UTR region of Chemokine, CXC Motif, Ligand 12 (CXCL12; SDF1) and VCAM1 mRNAs and reduce the expression of these proteins in HUVECs. Incubation of HUVECs with 10–50 µg/ml exosomes or miR-126 mimics resulted in decreased LAMA84 cells migration towards HUVEC conditioned medium and ability to adhesion to endothelial monolayer, in a dose dependent manner. Transendothelial migration assay showed that transendothelial migration of LAMA84 cells increased when HUVECs were treated with exosomes for 24 hours (63). According to their results Taverna *et al.*, 2014, speculated that; in the early phases of exosome treatment of HUVECs, VCAM1 expression levels and LAMA84 –HUVEC adhesion is elevated while the late phases of the exposure VCAM1 expression and adhesion capacity is decreased which allows LAMA84 cells migration towards chemoreacttant rich environment.

miRNA-126 targets pro-angiogenic proteins including Insulin-like Growth Factor Binding Protein 2 (IGFBP2), Phosphatidylinositol Transfer Protein Cytoplasmic 1 (PITPNC1) and c-Mer Tyrosine Kinase (MERTK) in metastatic breast cancer cell line CN34 LM1a. miRNA-126 frequently downregulated gene in most of the cancers. In this situation MERTK, IGFBP2 and PITPNC1 rescue from inhibition of miRNA-126. IGFBP2 secreted from metastatic cells induces endothelial cell chemotaxis and migration through IGF1/IGF1-R axis. MERTK receptors secreted from metastatic cells also contribute endothelial recruitment through blocking the binding of Growth Arrest-Specific 6 (GAS6) to endothelial MERTK receptors (64). miR-126 expression level is reduced in gastric carcinoma tissues obtained from patients. miR-126 binds to 3'UTR region of VEGF-A and regulate protein expression level of it. Ectopic overexpression of miR-126 in metastatic human gastric cancer cell line SGC-7901 results in reduced microvessel density and reduced VEGF-A expression (65). miR-126 expression level is lower in HSC3 oral cancer cell line which have high metastatic potential than in HSC4 cells compared with a low metastatic potential. Ectopic overexpression of miRNA-126 in HSC3 results in decreased expression of VEGF-A while inhibition of miRNA-126 in HSC4 results in elevated expression of VEGF-A. There is an inverse correlation was between the miR-126 expression levels and microvascular density, angiogenesis in HCC (66). miR-126-3p expression is down-regulated in HCC tumor tissues and a set of HCC cell lines. Microvascular density level is inversely correlated with miR-126-3p expression. Ectopic overexpression of miR-126-3p in HepG2 and SMMC-7721 cells reduce LRP6, PIK3R2 and Phospho-Akt expression levels and reduce micro-vessels and capillary-like structure formation ability of HUVECs. Suppression of miR-126-3p in BEL-7402 increased the angiogenesis ability of HUVECs (67).

According to the literature we can speculate that miRNA-126 is frequently downregulated in most of the tumor types which is inversely correlated with angiogenesis. Downregulation of miR-126 in cancer cells results in increased expression of LRP6 and PIK3R2 in tumor cells which further activates Wnt/beta-Catenin and PI3K/Akt/mTOR signaling

pathways. Activation of these pathways induce VEGF expression. Downregulation of miR126 also results in upregulation of other pro-angiogenic proteins including; IGFBP2, PITPNC1 and MERTK expression and secretion from metastatic cancer cells. This may results in activation of VEGF/VEGFR, IGFBP2/IGF1/IGF1R and c-MERTK/GAS6/MERTK cascades in endothelial cells and induces neovascularization. Selective packaging and enrichment of miRNA-126 into exosomes results in increased levels of miRNA-126 in the endothelial cells. miRNA-126 also contribute to migration and adhesion capability and angiogenesis through CXCL12/CXCR4 and VCAM1 modulation in a time dependent fashion.

6.3.5. Exosomal miR-21 mediated crosstalk

Treatment of HUVECs with K562 and LAMA84 exosomes incubated with Curcumin results in; decreased IL8 and MARCKS-Like Protein 1 (MARCKSL1) and VCAM1 expression in HUVECs, plasma membrane localisation of tight Junction marker Zona Occludens 1 (ZO1) and Vascular Endothelial Cadherin (VE-Cadherin) decrease, in endothelial capillary-like structure formation and motility. High levels of miR-21 in exosomes and HUVECs treated by them leads to inactivation of Ras Homolog Gene Family Member B (RhoB) and MARCKSL1 in HUVECs. Taverna *et al.*, 2015, showed that; miR-21 expression level is low in K562 and LAMA84 cells treated with Curcumin while miR-196b is high in these cells. On the contrary, in the exosomes secreted from these cells, miR-21 level is high as miR-126b is low. In Curcumin treated cells miR-21 downregulation results in decreased phospho-Akt and VEGF levels and an increase in PTEN expression in a dose-dependent manner. Increased miR-196b downregulates BCR/ABL mRNA in Curcumin treated CML cells. These molecular alterations correlated with reduced migration of K562 and LAMA84 cells towards in the medium (68-69).

In accordance with the results of Taverna *et al.* 2015, Liu LZ *et al.*, 2011 indicated that; ectopic overexpression of miR-21 in prostate cancer cell line DU145 results in decreased PTEN expression, increased expression of HIF-1 alpha and VEGF, elevation in microvessel formation and

angiogenesis. DU145 cells transfected miR-21 inhibitor increases PTEN expression while decrease phospho-Akt, phospho-ERK, HIF-1 alpha and VEGF expression levels, decrease angiogenesis (70). In breast cancer, miR-21 inhibition impairs angiogenesis by targeting HIF-1 alpha/VEGF/VEGFR2 signaling pathway (71). In these two studies mentioned above exosomal miR-21 content is not analysed. miR-21-5p is overexpressed in exosomes of thyroid carcinoma cell lines BCPAP and KTC-1 incubated under hypoxic contitions and in exosomes from sera of papillary thyroid cancer patients. Exosome mediated transportation of miR-21-5p suppress TGFBI and Collagen Type IV, Alpha-1 (COL4A1) expression while increase endothelial tube formation and angiogenesis in HUVECs (72).

Liu *et al.*, (2017) showed that; miR-21-5p is up-regulated in exosomes isolated serum of the NSCLC patients and associated with poor overall survival in patients (73). Exosomal miR-21 expression in the cerebrospinal fluids is also found to be elevated in glioma patients than that of the control cases (74). miR-196b-5p is overexpressed in exosomes isolated from sera of esophageal adenocarcinoma patients compared with tumor tissue and non-tumor samples (75).

These reports suggest that, upregulation of miR-21 level results in downregulation of PTEN, activation of PI3K/Akt/mTOR and Ras/Raf/MAPK pathways, increased production of HIF-1 alpha and VEGF in tumor cells. Exosomal miR-21 sorting and transportation may be differ between normoxic and hypoxic conditions. Activation of VEGF/VEGFR2 signalling in HUVECs induce angiogenesis, increased secretion from HUVECs. Activation of Rho/Rac/MARCKS axis in HUVECs under normoxic conditions and regulation of TGF-beta signalling under hypoxic condition also may contribute to angiogenesis.

After the unraveling the cell-cell communication of the CML cells and endothelial cells through a recent report showed that newly synthesized antiangiogenic peptide conjugated Gold Nanoparticles can interfere with VEGF/VEGFR axis and can be reduce neoangiogenesis (76).

6.4. Crosstalk between CML cells and BMSCs through exosomes

6.4.1. IL8-CXCR1/CXCR2 mediated crosstalk

Incubation of bone marrow-derived stromal cell line HS5 with 50 µg/ml of LAMA84 exosomes increase secretion of IL8 from HS5 into bone marrow microenvironment. Treatment of LAMA84 cells with 5 or 10 ng/ml of rIL8 for 48 h results in; increased expression and cell surface presentation of Chemokine, CXC Motif, Receptor 1-2 (CXCR1-CXCR2), increase in phospho-Akt levels increased adhesion ability to HS5 in a dose dependent manner and greater colony formation ability in methylcellulose. IL8 treatment also results in greater tumor formation in NOD/SCID mice in xenograft CML tumor model (77). These results show that; HS5 cells secrete IL8 after the treatment with LAMA84 exosomes or rIL8, IL8 binds CXCR1/CXCR2 receptors located on LAMA84 cell membrane, IL8 released from HS5 is able to modulate LAMA84 cells adhesion and motility properties, IL8 promotes the survival of LAMA84 cells through PI3K/Akt/mTOR signaling in both *in vivo* and *in vitro* models.

6.4.2. Exosomal AREG mediated crosstalk

Ligands of Epidermal Growth Factor <https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/epidermal-growth-factorReceptors> (EGFRs) include; Epidermal Growth Factor (EGF), Heparin-Binding EGF-Like Growth Factor (HBEGF), Transforming Growth Factor Alpha (TGF-α), Betacellulin (BTC), Amphiregulin (AREG), Epiregulin (EREG), Epithelial Mitogen (EPGN) and the Neuregulins (NRG 1–4). The EGFR family includes: EGFR (ErbB1, Her1), ErbB2 (Her2), ErbB3 (Her3) and ErbB4 (Her4). EGFR family signaling induces activation of RAS/MAPK, PLCγ/PKC, PI3K/Akt and JAK/STAT signaling pathways. Stimulation of BMSCs with EGFR ligands increases the production of growth and differentiation factors and cytokines including; VEGF, angiopoietin-2 (ANGPT2), Platelet-Derived Growth Factor BB (PDGF-BB), Granulocyte-Colony Stimulating Factor (G-CSF), Hepatocyte Growth Factor (HGF), and IL6, IL8 in BMSCs (78).

New diagnosed CML patients leukemia cells express EGFR ligands including; HBEGF,

EREG and AREG. LAMA84 cells and especially their exosomes overexpress AREG protein with respect to parental LAMA84 cells. Incubation of HS5 cells with LAMA84 derived exosomes results in; increased EGFR expression, activated EGFR signaling, increase in expression of Snail Family Transcriptional Repressor 1 (SNAIL) which is a downstream target of EGFR, increased levels of IL8 and MMP9 (SNAIL target genes), increased Annexin A2 (ANXA2) expression, increased adhesion of LAMA84 cells to stromal monolayer (79). AREG is overexpressed in multiple myeloma (MM) cells in a subset of patients and enriched in exosomes compared to parental MM cells (80). MM cell derived exosomes block osteogenic differentiation and increase the release of the pro-osteoclastogenic cytokines (IL6) from BMSCs through the activation of EGFR pathway. Treatment of BMSCs with MM cell derived exosomes increase the adhesion of MM1.S cells to the mesenchymal monolayer (81).

Based on the literature we can speculate that; CML cells and their exosomes express elevated levels of AREG. AREG containing exosomes induces EGFR expression and activation, EGFR signaling pathways induce SNAIL expression which is further induce IL8 and MMP9 synthesis and secretion from BMSCs, increased ANXA2 expression which induce active MMP formation, increased invasion and adhesion capacity, increased IL6 secretion leading to increased osteoclastogenic activity and blockage of osteoblastogenic differentiation.

6.4.3. Exosome mediated crosstalk through undefined mechanism

Incubation of human BMSCs with K562 exosomes influence the expression of various genes associated with osteoclast activation or osteoblast repression including; CXCL12, DKK1, Wnt5a, IL6, Tumor Necrosis Factor Alpha (TNF-α) and TGF-β depending on the exosome concentration and exposure time. Incubation of BMSCs with 50 µg/ml of K562 exosomes increase Nitric Oxide (NO) secretion from BMSCs. Incubation (48 and 72 hr) of BMSCs with K562-derived exosomes decrease the intracellular Reactive Oxygen Species (ROS) level in a dose-dependent manner (82). This increased osteoclastogenic activity leads to generation of extracellular milieu suitable for survival of CML cells.

There is a significant increase in transcript levels of ROS scavenging enzymes Manganese Superoxide Dismutase (MnSOD) and Catalase (CAT) and reduced ROS levels in CML patients BMSCs co-cultured with K562 CML cells. When BMSCs co-cultured with the K562 cells, they secreted significantly high levels of IL6 and TNF-alpha. This increase in IL6 was mediated by an increased phosphorylation of NFkB in BMSCs during its interaction with the CML cells (83). These findings are correlated with the findings of Jafarzadeh et al., 2019.

6.4.4. Exosomal miR-711 mediated crosstalk

Incubation of BMSCs with K562 exosomes results in exosome mediated transportation of miR-711 into BMSCs. miR722 have a binding site of 3'UTR region of CD44 mRNA. Transfection of BMSCs with miR-711 mimics lead also an increase in miR-711 levels which further decrease CD44 expression in BMSCs (84). Co-culturation of K562 cells and BMSCs with a direct contact between these two cell types results in downregulation of cell surface aminopeptidase-N (CD13), P Glycoprotein 1 (CD44), THY1 (CD90) and Fas (CD95) expression levels in BMSCs. Among these markers CD44 and CD90 are classical mesenchymal stromal cell surface biomarkers (83). Among these especially CD90 is reported to frequently downregulated in BMSCs isolated from leukemia cases. However exact role of this downregulation in leukemogenesis is unresolved (85-86).

6.4.5. Exosomal miR-320 mediated crosstalk

Incubation of human BMSCs with K562 and LAMA84 derived exosomes results in decreased Alkaline Phosphatase (ALP) and in decreased expression of osteogenic markers including; Runt-Related Transcription Factor 2; (RUNX2), Osteopontin (OPN) and Collagen Type I, Alpha-1 (COL1A1), CXCL12 and KITL in a dose-dependent manner. Several miRNAs are overexpressed in K562 and LAMA84 exosomes including; miR-320a/b/c/d, miR-3180-3p, miR-128-3p and miR-423-5p. RNA-binding Protein Heterogeneous Nuclear Ribonucleoprotein A1 (HNRNPA1) have been co-precipitate with miRNA-320a in cytoplasmic lysates

of K562 cells and ectopic overexpression of HNRNPA1 results an increase in exosomal miR-320 level but a decrease in intracellular compartments 3'-UTR regions of BCR/ABL and beta-Catenin mRNAs have a binding site for miR-320. HNRNPA1 mediated selective packaging of miR-320a into exosomes results in rescue of BCR/ABL mRNA from degradation in CML cells. In BMSCs degradation of beta-Catenin mRNA through miR-320a suppress maturation of osteoblasts (87). miR-320 is a tumor suppressor gene which leads to degradation of BCR/ABL mRNA in CML cells and downregulated in Imatinib resistant CML cases compared with imatinib sensitive patients (88). Recent studies have been shown that miR-320 is down-regulated in various types of cancers, including colon cancer, breast cancer, and acute myelogenous leukemia. miR-320 act as a tumor suppressor gene which repress tumor angiogenesis by targeting Neuropilin-1 (NRP1) in Oral Squamous Cell Carcinoma (OSCC) and frequently downregulated in OSCC (89). MiR-320 have a binding site at the 3'-UTR of Myeloid Cell Leukemia Sequence 1 (Mcl-1) mRNA which is a antiapoptotic activity. Downregulation of the miRNA-320 in human cervical cancer tissues results in upregulation of antiapoptotic protein Mcl-1 (90). miR-320a downregulate the expression levels of beta-Catenin, c-Myc, Cyclin D1 and DKK-1 in the human liver cancer cell line HepG2 cells. However, downregulation of miR-320 expression by using a anti-miR-320a siRNA results in upregulation of beta-Catenin, c-Myc, Cyclin D1 and DKK-1 expression (91). This results indicate that miR-320 is also a tumor suppressor for HCC. HNRNPA1 is an antiapoptotic protein which was reported to be upregulated in many tumors and markedly upregulated in refractory acute leukemia patients compared to sensitive patients (92).

According to the literature we can speculate that; CML cells express miR-320, CML exosomes include enriched miR-320, HNRPA1 selectively sort miR-320 into exosomes, reduced intracellular miR-320 results in rescue of BCR/ABL mRNA from degradation, increased antiapoptotic proteins Mcl-1, beta-Catenin, c-Myc, Cyclin D1 and DKK-1 expression in CML cells. Wnt/beta-Catenin signaling pathway is activated. Stabilized beta-Catenin translocate to the nucleus and generate a

CML derived exosome mediated signaling

Table 1. The effects of CML cell derived exosomal factors on gene expression in target cells

Secreting Cell	Exosomal Factor	Target Protein	Receiver Cell	Downregulated Proteins	Upregulated Proteins	Reference
LAMA84	TGF- beta1	TGFBR	LAMA84	BAD BAX PUMA	p-SMAD 2/3 p-Akt p-ERK NF-kB BCL-xL BCL2L2 Survivin	45
LAMA84R	IL8	IL8R	HUVEC	-	p-Erk½ p-Akt VCAM1 ICAM1 IL8	46
LAMA84 CML patients	IL8	IL8R	HUVEC	Cadherin Beta Catenin	p-MAPK p42/44 VCAM-1 ICAM IL8	47
K562	IL8	IL8R	HUVEC	-	p-Src	48
K562 ^{1%O2}	miR-210	EFNA3	HUVEC	EFNA3	-	49
K562	miR-92a	ITGA5	HUVEC	ITGA5	-	58
LAMA84	miRNA-126	CXCL12 VCAM1	HUVEC	CXCL12 VCAM1	-	63
Curcumin treated K562 and LAMA84	miR-21 miR-126b	Rho-B MARCKS	HUVEC	IL8 MARCKSL1 VCAM1 RhoB	Membraneous-ZO1 Membraneous-VE-Cadherin	69 68
LAMA84	Not defined	Not defined	HS5	-	IL8	77
Patient Sample LAMA84	AREG	EGFR	HS5	-	EGFR SNAIL IL8 MMP9 ANXA2	79
K562	Not defined	Not defined	Patient BMSCs sample	CXCL12 at 24h Wnt5a at 24h DKK1 at 72h IL6 at 72h TGF-beta at 24h ROS	CXCL12 at 48h Wnt5a at 48h DKK1 at 48h IL6 at 48h NO TNF- alpha at 72h	82
K562	miR-711	CD44	BMSCs	CD44	-	84
K562 and LAMA84	miR-320	Beta Catenin	Patient BMSCs sample	ALP RUNX2 Osteopontin OPN COL1A1 CXCL12 KITL	-	87

contd...

Table 1. Contd...

Secreting Cell	Exosomal Factor	Target Protein	Receiver Cell	Downregulated Proteins	Upregulated Proteins	Reference
K562	miR-92a-3p	C/EBP alpha	Patient ADSCs sample	C/EBP α PPAR γ FABP4 Adiponectin	-	94
HUVEC; Human Umbilical Vein Endothelial Cell, BMSCs; Bone Marrow Mesenchymal Stromal Cells; ADSCs; Adipose-Tissue Derived Stem Cells						

transcriptional complex with binding to TCF/LEF. miR-320 reduce the expression of beta-Catenin in BMSCs, reduce the maturation of osteoblasts in bone marrow, reduce the expression of osteogenic markers RUNX2, OPN and COL1A1.

6.4.6. Exosomal miR-92a-3p mediated crosstalk

During adipogenesis, several transcription factors including Peroxisome Proliferator-Activated Receptor-gamma (PPAR-gamma) and Cytidine-Cytidine-Adenosine-Adenosine-Thymidine (CCAAT)/Enhancer Binding Family of Proteins (C/EBP) play important roles in adipogenic differentiation of adipose tissue-derived mesenchymal stem cells (AMSCs) (93). Treatment of AMSC with K562-derived exosomes lead to decrease of lipid droplets formation, adipogenic transcription factor C/EBP-alpha mRNA expression and adipocyte-specific markers PPAR-gamma, Fatty Acide Binding Protein 4FABP4 and Adiponectin. miR-92a-3p expression was high in both K562 cells and its exosomes. C/EBP-alpha is one of the target genes of miR-92a-3p and coincubation of ADSCs with miR-92a-3p mimics transfected healthy donor exosomes results in lower adipogenic differentiation (94) However Wang *et al.*, 2008 reported that miR-17-92 cluster members (miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-92a) accelerate adipogenetic differentiation in mouse preadipocyte 3T3L1 cell line (95).

7. CONCLUSION REMARKS

Exosomes act as nano vehicles that transport signaling molecules from the CML cells to neighboring cells such as endothelial cells and bone marrow mesenchymal stromal cells.

Especially miRNA molecules transported through this way leads to alterations of reciever cells signaling pathways, gene expression pattern surface antigenic properties and protein secretion profiles. Growth factors, cytokines and other factors secreted from exosome reciever cell also act on CML cell characteristics and bone marrow niche. CML derived exosomes and their effects are summarized in Table 1. All of this alterations results in increased survival and growth, chemoresistance development, apoptosis inhibition, decreased adhesion to bone marrow matrix, increased migration and homing of quiescent CML stem cells in the bone marrow matrix in CML cells. This cell-cell communication results in an increase of neoangiogenesis from endothelial cells and suppression of adipogenic and osteoblastic activity and extracellular matrix remodeling through BMSCs. Especially activation of Hedgehog, Notch and Wnt signaling pathways in CML stem cells through cell-cell communication leads to chemoresistance, poor prognosis, recurrence of the disease after the drug discontinuation. It is obvious that there is a necessity for new therapeutic agents in addition to BCR/ABL TKIs to overcome these challenges targeting Hedgehog, Notch, Wnt and autophagy signaling pathways. May be near future loading of drugs, immunomodulator molecules, or miRNA or anti-miRNA transfer vectors into patient derived exosomes or exosomal mimics and targeting them to CML cells may come up as a useful approach to break this cell-cell communication.

8. ACKNOWLEDGMENTS

The authors do not declare any conflict of interest.

9. REFERENCES

1. J. D. Rowley. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*, 243 (5405), 290-3 (1973)
2. P. Trivedi, P. Varma, D. Patel, D. Ladani, D. Patel, M. Kazi, N. Patel, P. Patel: Clinical Implications of Simultaneous Occurrence of Variant Philadelphia Translocations in Chronic Myeloid Leukemia. *J Assoc Genet Technol*, 45 (2), 61-65 (2019)
3. C. Aydın, Z. Cetin, O. K. Yücel, U. İltar, O. Salim, S. Berker Karaüzüm: Additional Chromosomal Abnormalities and Variant Philadelphia Translocations in Newly Diagnosed Chronic Myeloid Leukemia; A Single Center Experience *Med J SDU*, 26(3), 320-327 (2019)
4. C. Aydın, Z. Cetin, O. Salim, O. K. Yucel, L. Undar, S. Berker Karauzum: Previously Unreported Chromosomal Aberrations of t(3;3)(q29;q23), t(4;11)-(q21;q23), and t(11;18)(q10;q10) in a Patient with Accelerated Phase Ph+ CML. *Case Rep Genet*, 582016 (2014)
DOI: 10.1155/2014/582016
5. P. A. Thompson, H. M. Kantarjian, J. E. Cortes: Diagnosis and Treatment of Chronic Myeloid Leukemia in 2015. *Mayo Clin Proc*, 90 (10), 1440-54 (2015)
DOI: 10.1016/j.mayocp.2015.08.010
6. R. Krishna Chandran, N. Geetha, K. M. Sakthivel, R. Suresh Kumar, K. M. N. Jagathnath Krishna, H. Sreedharan: Impact of Additional Chromosomal Aberrations on the Disease Progression of Chronic Myelogenous Leukemia. *Front Oncol*, 9, 88 (2019)
7. P. Gallipoli, A. Cook, S. Rhodes, L. Hopcroft, H. Wheadon, A. D. Whetton, H. G. Jørgensen, R. Bhatia, T. L. Holyoake: JAK2/STAT5 inhibition by nilotinib with ruxolitinib contributes to the elimination of CML CD34+ cells *in vitro* and *in vivo*. *Blood*, 124 (9), 1492-501 (2014)
DOI: 10.1182/blood-2013-12-545640
8. M. Bar-Natan, E.A. Nelson, M. Xiang, D.A. Frank. STAT signaling in the pathogenesis and treatment of myeloid malignancies. *JAKSTAT*, 1 (2), 55-64 (2012)
DOI: 10.4161/jkst.20006
9. B. T. Kaymaz, N. Selvi, C. Gündüz, C. Aktan, A. Dalmazrak, G. Saydam, B. Kosova: Repression of STAT3, STAT5A, and STAT5B expressions in chronic myelogenous leukemia cell line K-562 with unmodified or chemically modified siRNAs and induction of apoptosis. *Ann Hematol*, 92 (2), 151-62 (2013)
DOI: 10.1007/s00277-012-1575-2
10. L. Ma, Z. Xu, J. Wang, Z. Zhu, G. Lin, L. Jiang, X. Lu, C. Zou: Matrine inhibits BCR/ABL mediated ERK/MAPK pathway in human leukemia cells. *Oncotarget*, 8 (65), 108880-108889 (2017)
DOI: 10.18632/oncotarget.22353
11. J. Bertacchini, N. Heidari, L. Mediani, S. Capitani, M. Shahjahani, A. Ahmadzadeh, N. Saki: Targeting PI3K/AKT/mTOR network for treatment of leukemia. *Cell Mol Life Sci*. 72 (12), 2337-47 (2015)
DOI: 10.1007/s00018-015-1867-5
12. S. Dinner, L. C. Plataniias: Targeting the mTOR Pathway in Leukemia. *J Cell Biochem*, 117(8), 1745-52 (2016)
DOI: 10.1002/jcb.25559

DOI: 10.3389/fonc.2019.00088

13. R. Alves, A. C. Gonçalves, J. Jorge, J. Alves, A. Alves da Silva, P. Freitas-Tavares, J. M. Nascimento Costa, A. M. Almeida, A. B. Sarmento-Ribeiro: Everolimus in combination with Imatinib overcomes resistance in Chronic myeloid leukaemia. *Med Oncol*, 36 (3), 30 (2019)
DOI: 10.1007/s12032-019-1253-5
14. M. Varjosalo, J. Taipale. Hedgehog signaling. *J Cell Sci*, 120 (Pt 1), 3-6 (2007)
DOI: 10.1242/jcs.03309
15. J. Brechbiel, K. Miller-Moslin, A. A. Adjei: Crosstalk between hedgehog and other signaling pathways as a basis for combination therapies in cancer. *Cancer Treat Rev*, 40 (6), 750-9 (2014)
DOI: 10.1016/j.ctrv.2014.02.003
16. M. Pehlivan, C. Çalışkan, Z. Yüce, H. O. Sercan: Secreted Wnt antagonists in leukemia: A road yet to be paved. Wnt/ β -catenin signaling. *Leuk Res*, 69, 24-30 (2018)
DOI: 10.1016/j.leukres.2018.03.011
17. Y. Masamoto, M. Kurokawa: Targeting chronic myeloid leukemia stem cells: can transcriptional program be a druggable target for cancers? *Stem Cell Investig*, 5, 10 (2018)
DOI: 10.21037/sci.2018.03.05
18. E. Arrigoni, M. Del Re, S. Galimberti, G. Restante, E. Rofi, S. Crucitta, C. Baratè, M. Petrini, R. Danesi, A. Di Paolo: Concise Review: Chronic Myeloid Leukemia: Stem Cell Niche and Response to Pharmacologic Treatment. *Stem Cells Transl Med*, 7 (3), 305-314 (2018)
DOI: 10.1002/sctm.17-0175
19. K. Miyazono: Tumour promoting functions of TGF- β in CML-initiating cells. *J Biochem*, 152 (5), 383-5 (2012)
DOI: 10.1093/jb/mvs106
20. W. Hua, P. Ten Dijke, S. Kostidis, M. Giera, M. Hornsvelde: TGF β -induced metabolic reprogramming during epithelial-to-mesenchymal transition in cancer. *Cell Mol Life Sci*, (2019) (Epub ahead of print)
DOI: 10.1007/s00018-019-03398-6
21. X. Bai, M. Yi, Y. Jiao, Q. Chu, K. Wu: Blocking TGF- β Signaling To Enhance The Efficacy of Immune Checkpoint Inhibitor. *Onco Targets Ther*, 12, 9527-9538 (2019)
DOI: 10.2147/OTT.S224013
22. C. Sorrentino, A. Cuneo, G. Roti: Therapeutic Targeting of Notch Signaling Pathway in Hematological Malignancies. *Mediterr J Hematol Infect Dis*, 11 (1), e2019037 (2019)
DOI: 10.4084/MJHID.2019.037
23. F. Moradi, S. Babashah, M. Sadeghizadeh, A. Jalili, A. Hajifathali, H. Roshandel: Signaling pathways involved in chronic myeloid leukemia pathogenesis: The importance of targeting Musashi2-Numb signaling to eradicate leukemia stem cells. *Iran J Basic Med Sci*, 22 (6), 581-589 (2019)
DOI: 10.22038/ijbms.2019.31879.7666
24. M. Pelullo, S. Zema, F. Nardoza, S. Checquolo, I. Screpanti, D. Bellavia: Wnt, Notch, and TGF- β Pathways Impinge on Hedgehog Signaling Complexity: An Open Window on Cancer. *Front Genet*, 10, 711 (2019)
DOI: 10.3389/fgene.2019.00711

25. P. A. Pophali, M. M. Patnaik: The Role of New Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia. *Cancer J*, 22 (1), 40-50 (2016)
DOI: 10.1097/PPO.0000000000000165
26. E. Jabbour, H. Kantarjian: Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *Am J Hematol*, 93 (3), 442-459. (2018)
DOI: 10.1002/ajh.25011
27. K. K. Chen, T. F. Du, K. S. Wu, W. Yang: First-line treatment strategies for newly diagnosed chronic myeloid leukemia: a network meta-analysis. *Cancer Manag Res*, 10, 3891-3910 (2018)
DOI: 10.2147/CMAR.S177566
28. E. Jabbour, J. Cortes, H. Kantarjian: Nilotinib for the treatment of chronic myeloid leukemia: An evidence-based review. *Core Evid*, 4: 207–213 (2009)
DOI: 10.2147/ce.s6003
29. D. G. Aguilera, A. M. Tsimberidou: Dasatinib in chronic myeloid leukemia: a review. *Ther Clin Risk Manag*, 5, 281–289 (2009)
DOI: 10.2147/tcrm.s3425
30. C. Gambacorti-Passerini, T. H. Brümmendorf, G. W. Kim, A. G. Turkina, A. Masszi, S. Assouline, S. Durrant, H. M. Kantarjian, H. J. Khoury, A. Zaritskey, Z. X. Shen, J. Jin, E. Vellenga, R. Pasquini, V. Mathews, F. Cervantes, N. Besson, K. Turnbull, E. Leip, V. Kelly, J. E. Cortes: Bosutinib efficacy and safety in chronic phase chronic myeloid leukemia after imatinib resistance or intolerance: Minimum 24-month follow-up. *Am J Hematol*, 89 (7), 732–742 (2014)
DOI: 10.1002/ajh.23728
31. A. E. Eskazan, D. Keskin: Radotinib and its clinical potential in chronic-phase chronic myeloid leukemia patients: an update. *Ther Adv Hematol*, 8 (9), 237–243 (2017)
DOI: 10.1177/2040620717719851
32. O. Frankfurt, J. D. Licht: Ponatinib--a step forward in overcoming resistance in chronic myeloid leukemia. *Clin Cancer Res*.19 (21), 5828-34 (2013)
DOI: 10.1158/1078-0432.CCR-13-0258
33. M. Yaghmaie, C. C. Yeung: Molecular Mechanisms of Resistance to Tyrosine Kinase Inhibitors. *Curr Hematol Malig Rep*, 14 (5), 395-404 (2019)
DOI: 10.1007/s11899-019-00543-7
34. F. Loscocco, G. Visani, S. Galimberti, A. Curti, A. Isidori: BCR-ABL Independent Mechanisms of Resistance in Chronic Myeloid Leukemia. *Front Oncol*, 9, 939. (2019)
DOI: 10.3389/fonc.2019.00939
35. L. L. Yu, J. Zhu, J. X. Liu, F. Jiang, W. K. Ni, L. S. Qu, R. Z. Ni, C. H. Lu, M. B. Xiao: A Comparison of Traditional and Novel Methods for the Separation of Exosomes from Human Samples. *Biomed Res Int*, 2018, 3634563 (2018)
DOI: 10.1155/2018/3634563
36. F. X. Li, J. J. Liu, F. Xu, X. Lin, J. Y. Zhong, F. Wu, L. Q. Yuan: Role of tumor-derived exosomes in bone metastasis. *Oncol Lett*, 18 (4), 3935-3945 (2019)
DOI: 10.3892/ol.2019.10776
37. S. Anand, M. Samuel, S. Kumar, S. Mathivanan: Ticket to a bubble ride: Cargo sorting into exosomes and extracellular vesicles. *Biochim Biophys Acta Proteins Proteom*, 1867 (12), 140203 (2019)

- DOI: 10.1016/j.bbapap.2019.02.005
38. K. Kluszczyńska, L. Czernek, W. Cypryk, Ł. Pęczek, M. Düşler: Methods for the determination of the purity of exosomes. *Curr Pharm Des*, 25 (42), 4464-4485 (2019)
DOI: 10.2174/13816128256-66191206162712
 39. T. Soares Martins, J. Catita, I. Martins Rosa, O A. B. da Cruz E Silva, A.G. Henriques: Exosome isolation from distinct biofluids using precipitation and column-based approaches. *PLoS One*, 13 (6), e0198820 (2018)
DOI: 10.1371/journal.pone.0198820
 40. L. M. Doyle, M. Z. Wang: Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells*, 8 (7), pii: E727 (2019)
DOI: 10.3390/cells8070727
 41. L. Mashouri, H. Yousefi, A. R. Aref, A. M. Ahadi, F. Molaei, S. K. Alahari: Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer*, 18 (1), 75 (2019)
DOI: 10.1186/s12943-019-0991-5
 42. R. Sahebi, H. Langari, Z. Fathinezhad, Z. Bahari Sani, A. Avan, M. Ghayour Mobarhan, M. Rezayi: Exosomes: New insights into cancer mechanisms. *J Cell Biochem*, 121 (1), 7-16 (2020)
DOI: 10.1002/jcb.29120
 43. X. Zhang, C. Y. J. B. X. L. W. G. Z. Y. Zhou: The function of tumor-derived exosomes. *J BUON*, 24 (3), 897-904 (2019)
 44. D. Q. Feng, B. Huang, J. Li, J. Liu, X. M. Chen, Y. M. Xu, X. Chen, H. B. Zhang, L. H. Hu, X. Z. Wang: Selective miRNA Expression Profile in Chronic Myeloid Leukemia K562 Cell-derived Exosomes. *Asian Pac J Cancer Prev*, 14 (12), 7501-8 (2013)
DOI: 10.7314/APJCP.2013.14.12.7501
 45. S. Raimondo, L. Saieva, C. Corrado, S. Fontana, A. Flugy, A. Rizzo, G. De Leo, R. Alessandro: Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism. *Cell Commun Signal*, 13, 8 (2015)
DOI: 10.1186/s12964-015-0086-x
 46. C. Corrado, A. M. Flugy, S. Taverna, S. Raimondo, G. Guggino, R. Karmali, G. De Leo, R. Alessandro: Carboxyamidotriazole-ototate inhibits the growth of imatinib-resistant chronic myeloid leukaemia cells and modulates exosomes-stimulated angiogenesis. *PLoS One*, 7 (8), e42310. (2012)
DOI: 10.1371/journal.pone.0042310
 47. S. Taverna, A. Flugy, L. Saieva, E. C. Kohn, A. Santoro, S. Meraviglia, G. De Leo, R. Alessandro: Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis. *Int J Cancer*, 130 (9), 2033-43 (2012)
DOI: 10.1002/ijc.26217
 48. M. Mineo, S. H. Garfield, S. Taverna, A. Flugy, G. De Leo, R. Alessandro, E. C. Kohn: Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a src-dependent fashion. *Angiogenesis*, 15 (1), 33-45 (2012)
DOI: 10.1007/s10456-011-9241-1
 49. H. Tadokoro, T. Umezu, K. Ohyashiki, T. Hirano, J. H. Ohyashiki: Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. *J Biol Chem*, 288 (48), 34343-51 (2013)

- DOI: 10.1074/jbc.M113.480822
50. H. Cui, B. Seubert, E. Stahl, H. Dietz, U. Reuning, L. Moreno-Leon, M. Ilie, P. Hofman, H. Nagase, B. Mari, A. Krüger: Tissue inhibitor of metalloproteinases-1 induces a pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes. *Oncogene*, 34 (28), 3640-50 (2015)
DOI: 10.1038/onc.2014.300
51. H. W. King, M. Z. Michael, J. M. Gleadle: Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer*, 12, 421 (2012)
DOI: 10.1186/1471-2407-12-421
52. J. Chen, R. Xu, J. Xia, J. Huang, B. Su, S. Wang: Aspirin inhibits hypoxia-mediated lung cancer cell stemness and exosome function. *Pathol Res Pract*, 215 (6), 152379 (2019)
DOI: 10.1016/j.prp.2019.03.008
53. X. J. Lin, J. H. Fang, X. J. Yang, C. Zhang, Y. Yuan, L. Zheng, S. M. Zhuang: Hepatocellular Carcinoma Cell-Secreted Exosomal MicroRNA-210 Promotes Angiogenesis *In vitro* and *In vivo*, 11, 243-252 (2018)
DOI: 10.1016/j.omtn.2018.02.014
54. N. Kosaka, H. Iguchi, K. Hagiwara, Y. Yoshioka, F. Takeshita, T. Ochiya: Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. *J Biol Chem*, 288 (15), 10849-59 (2013)
DOI: 10.1074/jbc.M112.446831
55. K. Dang, K. A. Myers: The role of hypoxia-induced miR-210 in cancer progression. *Int J Mol Sci*, 16 (3), 6353-72 (2015)
56. Y. Yang, J. Zhang, T. Xia, G. Li, T. Tian, M. Wang, R. Wang, L. Zhao, Y. Yang, K. Lan, W. Zhou: MicroRNA-210 promotes cancer angiogenesis by targeting fibroblast growth factor receptor-like 1 in hepatocellular carcinoma. *Oncol Rep*, 36 (5), 2553-2562 (2016)
DOI: 10.3892/or.2016.5129
57. W. J. Cao, J. D. Rosenblat, N. C. Roth, M. A. Kuliszewski, P. N. Matkar, D. Rudenko, C. Liao, P. J. Lee, H. Leong-Poi: Therapeutic Angiogenesis by Ultrasound-Mediated MicroRNA-126-3p Delivery. *Arterioscler Thromb Vasc Biol*, 35 (11), 2401-11 (2015)
DOI: 10.1161/ATVBAHA.115.306506
58. T. Umezu, K. Ohyashiki, M. Kuroda, J. H. Ohyashiki: Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene*, 32 (22), 2747-55 (2013)
DOI: 10.1038/onc.2012.295
59. N. Yamada, Y. Nakagawa, N. Tsujimura, M. Kumazaki, S. Noguchi, T. Mori, I. Hirata, K. Maruo, Y. Akao: Role of intracellular and extracellular microrna-92a in colorectal cancer. *Transl Oncol*, 6, 482-492 (2013)
DOI: 10.1593/tlo.13280
60. N. O. Yamada, K. Heishima, Y. Akao, T. Senda: Extracellular Vesicles Containing MicroRNA-92a-3p Facilitate Partial Endothelial-Mesenchymal Transition and Angiogenesis in Endothelial Cells. *Int J Mol Sci*, 20 (18), pii: E4406 (2019)
DOI: 10.3390/ijms20184406
61. A. Chamorro-Jorganes, M. Y. Lee, E. Araldi, S. Landskroner-Eiger, M. Fernández-Fuertes, M. Sahraei, M. Quiles

- Del Rey, C. van Solingen, J. Yu, C. Fernández-Hernando, W. C. Sessa, Y. Suárez: VEGF-Induced Expression of miR-17-92 Cluster in Endothelial Cells Is Mediated by ERK/ELK1 Activation and Regulates Angiogenesis. *Circ Res*, 118 (1), 38-47 (2016)
DOI: 10.1161/CIRCRESAHA.115.307408
62. K. Kochan-Jamrozy, J. Króliczewski, A. Moszyńska, J. F. Collawn, R. Bartoszewski: miRNA networks modulate human endothelial cell adaptation to cyclic hypoxia. *Cell Signal*, 54, 150-160 (2019)
DOI: 10.1016/j.cellsig.2018.11.020
63. S. Taverna, V. Amodeo, L. Saieva, A. Russo, M. Giallombardo, G. De Leo, R. Alessandro: Exosomal shuttling of miR-126 in endothelial cells modulates adhesive and migratory abilities of chronic myelogenous leukemia cells. *Mol Cancer*, 13, 169 (2014)
DOI: 10.1186/1476-4598-13-169
64. K. J. Png, N. Halberg, M. Yoshida, S. F. Tavazoie: A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature*, 481 (7380), 190-4 (2011)
DOI: 10.1038/nature10661
65. H. Chen, L. Li, S. Wang, Y. Lei, Q. Ge, N. Lv, X. Zhou, C. Chen: Reduced miR-126 expression facilitates angiogenesis of gastric cancer through its regulation on VEGF-A. *Oncotarget*, 5 (23), 11873-85 (2014)
DOI: 10.18632/oncotarget.2662
66. T. Sasahira, M. Kurihara, U. K. Bhawal, N. Ueda, T. Shimomoto, K. Yamamoto, T. Kirita, H. Kuniyasu: Downregulation of miR-126 induces angiogenesis and lymphangiogenesis by activation of VEGF-A in oral cancer. *Br J Cancer*, 107 (4), 700-6 (2012)
DOI: 10.1038/bjc.2012.330
67. C. Du, Z. Lv, L. Cao, C. Ding, O. A. Gyabaah, H. Xie, L. Zhou, J. Wu, S. Zheng: MiR-126-3p suppresses tumor metastasis and angiogenesis of hepatocellular carcinoma by targeting LRP6 and PIK3R2. *J Transl Med*, 12, 259 (2014)
DOI: 10.1186/s12967-014-0259-1
68. S. Taverna, S. Fontana, F. Monteleone, M. Pucci, L. Saieva, V. De Caro, V. G. Cardinale, M. Giallombardo, E. Vicario, C. Rolfo, G. D. Leo, R. Alessandro: Curcumin modulates chronic myelogenous leukemia exosomes composition and affects angiogenic phenotype via exosomal miR-21. *Oncotarget*, 7 (21), 30420-39 (2016)
DOI: 10.18632/oncotarget.8483
69. S. Taverna, M. Giallombardo, M. Pucci, A. Flugy, M. Manno, S. Raccosta, C. Rolfo, G. De Leo, R. Alessandro: Curcumin inhibits *in vitro* and *in vivo* chronic myelogenous leukemia cells growth: a possible role for exosomal disposal of miR-21. *Oncotarget*, 6 (26), 21918-33 (2015)
DOI: 10.18632/oncotarget.4204
70. L. Z. Liu, C. Li, Q. Chen, Y. Jing, R. Carpenter, Y. Jiang, H. F. Kung, L. Lai, B. H. Jiang, N. Liu, S. Zang, Y. Liu, Y. Wang, W. Li, Q. Liu, M. Ji, D. Ma, C. Ji: FZD7 regulates BMSCs-mediated protection of CML cells. *Oncotarget*, 7 (5), 6175-87 (2016)
DOI: 10.18632/oncotarget.6742
71. D. Zhao, Y. Tu, L. Wan, L. Bu, T. Huang, X. Sun, K. Wang, B. Shen: *In vivo* monitoring of angiogenesis inhibition via

- down-regulation of mir-21 in a VEGFR2-luc murine breast cancer model using bioluminescent imaging. *PLoS One*, 8 (8), e71472 (2013)
DOI: 10.1371/journal.pone.0071472
72. F. Wu, F. Li, X. Lin, F. Xu, R. Cui, J. Zhong, T. Zhu, S. K. Shan, X. B. Liao, L. Yuan, Z. Mo: Exosomes increased angiogenesis in papillary thyroid cancer microenvironment. *Endocr Relat Cancer*, 26(5), 525-538 (2019)
DOI: 10.1530/ERC-19-0008
 73. Q. Liu, Z. Yu, S. Yuan, W. Xie, C. Li, Z. Hu, Y. Xiang, N. Wu, L. Wu, L. Bai, Y. Li: Circulating exosomal microRNAs as prognostic biomarkers for non-small-cell lung cancer. *Oncotarget*, 8 (8), 13048-13058 (2017)
DOI: 10.18632/oncotarget.14369
 74. R. Shi, P. Y. Wang, X. Y. Li, J. X. Chen, Y. Li, X. Z. Zhang, C. G. Zhang, T. Jiang, W. B. Li, W. Ding, S. J. Cheng: Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. *Oncotarget*, 6 (29), 26971-81 (2015)
DOI: 10.18632/oncotarget.4699
 75. U. Warnecke-Eberz, S. H. Chon, A. H. Hölscher, U. Drebber, E. Bollschweiler: Exosomal onco-miRs from serum of patients with adenocarcinoma of the esophagus: comparison of miRNA profiles of exosomes and matching tumor. *Tumour Biol*, 36 (6), 4643-53 (2015)
DOI: 10.1007/s13277-015-3112-0
 76. C. Roma-Rodrigues, A. R. Fernandes, P. V. Baptista: Counteracting the effect of leukemia exosomes by antiangiogenic gold nanoparticles. *Int J Nanomedicine*, 14, 6843-6854 (2019)
DOI: 10.2147/IJN.S215711
 77. C. Corrado, S. Raimondo, L. Saieva, A. M. Flugy, G. De Leo, R. Alessandro: 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*, 348 (1-2), 71-6 (2014)
DOI: 10.1016/j.canlet.2014.03.009
 78. S. Müller-Deubert, L. Seefried, M. Krug, F. Jakob, R. Ebert: Epidermal growth factor as a mechanosensitizer in human bone marrow stromal cells. *Stem Cell Res*, 24, 69-76 (2017)
DOI: 10.1016/j.scr.2017.08.012
 79. C. Corrado, L. Saieva, S. Raimondo, A. Santoro, G. De Leo, R. Alessandro: Chronic myelogenous leukaemia exosomes modulate bone marrow microenvironment through activation of epidermal growth factor receptor. *J Cell Mol Med*, 20 (10), 1829-39 (2016)
DOI: 10.1111/jcmm.12873
 80. K. Mahtouk, D. Hose, T. Rème, J. De Vos, M. Jourdan, J. Moreaux, G. Fiol, M. Raab, E. Jourdan, V. Grau, M. Moos, H. Goldschmidt, M. Baudard, J. F. Rossi, F. W. Cremer, B. Klein: Expression of EGF-family receptors and amphiregulin in multiple myeloma. Amphiregulin is a growth factor for myeloma cells. *Oncogene*, 24 (21), 3512-24 (2005)
DOI: 10.1038/sj.onc.1208536
 81. S. Raimondo, L. Saieva, E. Vicario, M. Pucci, D. Toscani, M. Manno, S. Raccosta, N. Giuliani, R. Alessandro: Multiple myeloma-derived exosomes are enriched of amphiregulin (AREG) and activate the epidermal growth factor pathway in the bone microenvironment

- leading to osteoclastogenesis. *J Hematol Oncol*, 12 (1), 2 (2019)
DOI: 10.1186/s13045-018-0689-y
82. N. Jafarzadeh, Z. Safari, M. Pornour, N. Amirizadeh, M. Forouzandeh Moghadam, M. Sadeghizadeh: Alteration of cellular and immune-related properties of bone marrow mesenchymal stem cells and macrophages by K562 chronic myeloid leukemia cell derived exosomes. *J Cell Physio*, 234 (4), 3697-3710 (2019)
DOI: 10.1002/jcp.27142
83. A. Kumar, T. Anand, J. Bhattacharyya, A. Sharma, B. G. Jaganathan: K562 chronic myeloid leukemia cells modify osteogenic differentiation and gene expression of bone marrow stromal cells. *J Cell Commun Signal*, 12 (2), 441–450 (2018)
DOI: 10.1007/s12079-017-0412-8
84. Y. H. Jiang, J. Liu, J. Lin, S. Q. Li, Y. M. Xu, Q. H. Min, Q. H. Zhong, F. Sun, J. Li, X. H. You, K. L. Liao, T. Y. Qin, C. Zhao, B. Huang, X. Z. Wang: K562 cell-derived exosomes suppress the adhesive function of bone marrow mesenchymal stem cells via delivery of miR-711. *Biochem Biophys Res Commun*, 521(3), 584-589 (2020)
DOI: 10.1016/j.bbrc.2019.10.096
85. B. G. Jaganathan, V. Tisato, T. Vulliamy, I. Dokal, J. Marsh, F. Dazzi, D. Bonnet: Effects of MSC co-injection on the reconstitution of aplastic anemia patient following hematopoietic stem cell transplantation. *Leukemia*, 24 (10), 1791–1795 (2010)
DOI: 10.1038/leu.2010.164
86. D. Campioni, R. Rizzo, M. Stignani, L. Melchiorri, L. Ferrari, S. Moretti, A. Russo, G. P. Bagnara, L. Bonsi, F. Alviano, G. Lanzoni, A. Cuneo, O. R. Baricordi, F. Lanza: A decreased positivity for CD90 on human mesenchymal stromal cells (MSCs) is associated with a loss of immunosuppressive activity by MSCs. *Cytometry B Clin Cyto.* 76B (3), 225–230 (2009)
DOI: 10.1002/cyto.b.20461
87. X. Gao, Z. Wan, M. Wei, Y. Dong, Y. Zhao, X. Chen, Z. Li, W. Qin, G. Yang, L. Liu: Chronic myelogenous leukemia cells remodel the bone marrow niche via exosome-mediated transfer of miR-320. *Theranostics*, 9 (19), 5642-5656 (2019)
DOI: 10.7150/thno.34813
88. D. Jurkovicova, R. Lukackova, M. Magyerkova, L. Kulcsar, M. Krivjanska, V. Krivjansky, M. Chovanec: microRNA expression profiling as supportive diagnostic and therapy prediction tool in chronic myeloid leukemia. *Neoplasma*, 62 (6), 949-58 (2015)
DOI: 10.4149/neo_2015_115
89. Y. Y. Wu, Y. L. Chen, Y. C. Jao, I. S. Hsieh, K.C. Chang, T.M. Hong: miR-320 regulates tumor angiogenesis driven by vascular endothelial cells in oral cancer by silencing neuropilin 1. *Angiogenesis*, 17(1), 247-60 (2014)
DOI: 10.1007/s10456-013-9394-1
90. T. Zhang, P. Zou, T. Wang, J. Xiang, J. Cheng, D. Chen, J. Zhou: Down-regulation of miR-320 associated with cancer progression and cell apoptosis via targeting Mcl-1 in cervical cancer. *Tumour Biol*, 37 (7), 8931-40 (2016)
DOI: 10.1007/s13277-015-4771-6
91. C. Lu, Z. Liao, M. Cai, G. Zhan: MicroRNA-320a downregulation mediates human liver cancer cell proliferation through the Wnt/ β -catenin signaling pathway. *Oncol Lett*, 13 (2), 573–578 (2017)

DOI: 10.3892/ol.2016.5479

92. Q. Wei, Y. Li, L. Chen, L. Zhang, X. He, X. Fu, K. Ying, J. Huang, Q. Chen, Y. Xie, Y. Mao: Genes differentially expressed in responsive and refractory acute leukemia. *Front Biosci*, 11, 977-82 (2006)

DOI: 10.2741/1854

93. Y. Wang, L. Lin, Y. Huang, J. Sun, X. Wang, P. Wang: MicroRNA-138 Suppresses Adipogenic Differentiation in Human Adipose Tissue-Derived Mesenchymal Stem Cells by Targeting Lipoprotein Lipase. *Yonsei Med J*, 60 (12), 1187-1194 (2019)

DOI: 10.3349/ymj.2019.60.12.1187

94. Z. Wan, X. Chen, X. Gao, Y. Dong, Y. Zhao, M. Wei, W. Fan, G. Yang, L. Liu: Chronic myeloid leukemia-derived exosomes attenuate adipogenesis of adipose derived mesenchymal stem cells via transporting miR-92a-3p. *J Cell Physiol*, 234 (11), 21274-21283 (2019)

DOI: 10.1002/jcp.28732

95. Q. Wang, Y. C. Li, J. Wang, J. Kong, Y. Qi, R. J. Quigg, X. Li: miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. *Proc Natl Acad Sci USA*, 105 (8), 2889-2894 (2008)

DOI: 10.1073/pnas.0800178105

Abbreviations: 4EBP1: Eukaryotic Translation Initiation Factor 4E-Binding Protein 1, ADAM: A-Disintegrin and Metalloprotease, ANGPT2: Angiopoietin-2, ANXA2: Annexin A2, APC: Adenomatous Polyposis Coli, AREG: Amphiregulin, AXIN: Axis Inhibitor, BAD: BCL2-Associated Agonist of Cell Death, BAX: BCL2-Associated X, BCL2L2: BCL2-Like 2, BCL-xl: BCL2-Related Protein, Long Isoform, BCR: Breakpoint Cluster

Region, BTC: Betacellulin, C/EBP: CCAAT/Enhancer Binding Proteins, CAT: Catalase, CK1: Creatin Kinase 1, CLDN11: Claudin 11, CML: Chronic Myeloid Leukemia, COL1A1: Collagen Type I, Alpha-1, COX-2: Cyclooxygenase-2, CTO: Carboxyamidotriazole Orotate, CXCL12: Chemokine, CXC Motif, Ligand 12, Delta: Ligand of the Delta-like, Dhh: Desert Hedgehog, DLS: Dynamic Light Scattering, Dvl: Dishevelled, EDN1: Endothelin 1, EDNRB: Endothelin-B Receptor, EFNA3: Ephrin A3, EGF: Epidermal Growth Factor, EGFR: Epidermal Growth Factor Receptor, EPGN: Epithelial Mitogen, EphA2: Ephrin Receptor A2, EREG: Epiregulin, ERK: Extracellular Signal Regulated Kinase, ESCRT: Endosomal Sorting Complexes Required for Transport, FAK: Focal Adhesion Kinase, FDA: Food and Drug Administration, FLT3: FMS-Related Tyrosine Kinase 3, FOXO: Forkhead Box O, Fzd: Frizzled, GAS6: Growth Arrest-Specific 6, G-CSF: Granulocyte-Colony Stimulating Factor, Grb2: Growth Factor Receptor Bound protein 2, GSK-3: Glycogen Synthase Kinase 3, HBEGF: Heparin-Binding EGF-Like Growth Factor, HES: Hairy/Enhancer of Split, HEY: Hairy/Enhancer of Split-Related with YRPW Motif, HGF: Hepatocyte Growth Factor, HIF-1 alpha: Hypoxia Inducible Factor 1 Alpha, HNRNPA1: Heterogeneous Nuclear Ribonucleoprotein A1, HSC70: Heat-Shock Cognate Protein 70, HSP90: Heat Shock Protein 90, ICAM-1: Intercellular Adhesion Molecule-1, IGFBP2: Insulin-like Growth Factor Binding Protein 2, PITPNC1: Phosphatidylinositol Transfer Protein Cytoplasmic 1 Ihh: Indian Hedgehog, ITGA5: Integrin α 5, JAG: Jagged, JAK: Janus Kinase JNK: c-Jun N-terminal Kinase, Klf4: Krueppel-Like Factor 4, LRP5/6: Low-Density Lipoprotein Receptor-Related Protein, MAML1-3: Mastermind-Like, MARCKSL1: MARCKS-Like Protein 1, MERTK: c-Mer Tyrosine Kinase, MMP9: Matrix Metalloproteinase 9, MnSOD: Manganese Superoxide Dismutase, mSIN1: SAPK Interacting Protein 1, mTOR: Mammalian Target of Rapamycin, NECD: Notch Extracellular

Domain, NF- κ B: Nuclear Factor-kappa B, NICD: Notch Intracellular Domain, NO: Nitric Oxide, NOD/SCID: Non Obese Diabetic/ Severe Combine Immudodeficient, NRG: Neuregulin, NRP1: Neuropilin-1, nSMase: Sphingomyelinase, NTA: Nanoparticle Tracking Analysis, OPN: Osteopontin, OSCC: Oral Squamous Cell Carcinoma, PDGF-BB: Platelet-Derived Growth Factor BB, PDGFR: Platelet-Derived Growth Factor Receptor, PDK1: Phosphoinositide-Dependent Kinase 1, Ph: Philadelphia, PI3K: Phosphatidylinositol 3-Kinase, PIP2: Phosphatidylinositol 4,5-bisphosphate, PIP3: Phosphatidylinositol 3,4,5-trisphosphate, PIPNC1: Phosphatidylinositol Transfer Protein Cytoplasmic 1, PP2A: Protein Phosphatase 2A, PPAR γ : Peroxisome Proliferator-Activated Receptor- γ , Prop1: Paired Like Homeobox-1, Protor: Protein Observed with Rictor1, PS: Presenilin, Ptc: Patched1, PTEN: Phosphatase and Tensin Homologue, PUMA: p53-Upregulated Modulator of Apoptosis, RAPTOR: Regulatory Associated Protein of mTOR, RBPJ: Recombination Signal-Binding Protein for Immunoglobulin Kappa J Region, RET: Rearranged During Transfection, Rheb: Ras Homolog Enriched in Brain, RhoB: Ras Homolog Gene Family Member B, RICTOR: Rapamycin-Insensitive Companion of mTOR, rpS6: S6 Ribosomal Protein, RUNX2: Runt-Related Transcription Factor 2, S6K: S6 Kinase, SEM: Scanning Electron Microscopy, Shh: Sonic Hedgehog, sKITL: Kit Ligand, SMAD: Sma-and Mad-Related Protein Smo: Smoothed, SOS: Son of Sevenless, STAT: Signal Transducer and Activator of Transcription, TEM: Transmission Electron Microscopy, TGF: Transforming Growth Factor, THBS1: Thrombospondin 1, TIMP-1: Tissue Inhibitor of Metalloproteinase 1, TKIs: Tyrosine Kinase Inhibitors, TNF-alpha: Tumor Necrosis Factor Alpha, tRPS: Tunable Resistive Pulse Sensing, TSC: Tuberous Sclerosis, TSG101: Tumor Susceptibility Gene 101, VCAM1: Vascular Cell Adhesion Molecule 1,

VEGF: Vascular Endothelial Growth Factor, VEGFR: Vascular Endothelial Growth Factor Receptor

Key Words: Chronic Myeloid Leukemia, CML, Exosomes, mi-RNA, angiogenesis, adhesion, migration, drug resistance, bone marrow mesenchymal stromal cells, BMSCs, CML leukemia stem cells, Review

Send correspondence to: Zafer Cetin, Sanko University, School of Medicine, Department of Medical Biology, Gazimuhtar Pasa Bulvarı No:36 27090 Sehitkamil, Gaziantep, Turkey, Tel: 03422116556, Fax: 03422116566, E-mail: alexg@mail.ntua.gr