rhuGM-CSF (Sargramostim; Leukine, Bayer HealthCare Pharmaceuticals). However, because of an inferior $CD34^+$ yield and marginal clinical benefits in this setting, its use as a mobilizing agent remains limited.[3] Plerixafor (Mozobil; AMD3100, Genzyme Corp) is a bicyclam derivative that reversibly inhibits the binding of stromal cell–derived factor-1 (SDF-1/CXCL12), a chemokine, to its receptor, CXCR4.[7] Plerixafor is thought to act as an antagonist (or possibly a partial agonist) of the α chemokine receptor CXCR4, and an allosteric agonist of CXCR7. In the autologous setting, the addition of Plerixafor to the G-CSF regimen for PBSC mobilization in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) appears to increase $CD34^+$ cell collection; resulting in fewer days of apheresis, and a higher proportion of patients proceeding to transplantation than with G-CSF alone. This suggests that regimens that mobilize PBSCs in donors who normally fail mobilization are of practical clinical benefit.

The role for the SDF-1/CXCR4 axis in retention of HSCs/HPCs in the BM under steady-state conditions is widely accepted. However, its role in stem cell trafficking, the processes of homing or mobilization, has needed additional clarification. At its most basic level, the question that persisted was; "If the SDF-1 gradient across the BM-PB barrier drives the homing of HSCs/HPCs into the BM during HSCT, what drives the release of cells from the BM microenvironment into the PB when the SDF-1/CXCR4 axis is disrupted?" Mechanistically, it appears that the homing of HSCs/HPCs from the PB into the BM is facilitated by the SDF-1/CXCR4 axis and that disruption of this axis results in the release of HSCs/HPCs. However, the model by which the SDF-1/CXCR4 axis changes during mobilization, thereby facilitating the release of HSCs/HPCs is unclear. It is in this context that the S1P active phospholipid has entered the discussion as a novel chemoattractant for HSCs/HPCs and a possible explanation for the mechanism behind release of HSCs/HPCs into PB. S1P, a product of 2 sphingosine kinases (SK1 and SK2), interacts with at least 5 G-protein–coupled seven-transmembrane–spanning receptors, $S1P_{1\text{-}5}$, on the surface of target cells to induce cell migration. HSCs have been shown to express S1P receptors and migrate in response to S1P.[8]

Here, Juarez et al studied the role of the $S1P/S1P_1$ axis in PBSC mobilization and determined that (1) pharmacologic inhibition of the $S1P/S1P_1$ axis (using FTY720; Novartis) or use of $S1P_{1\text{-}/\text{-}}$ mice inhibits CXCR4 antagonist (AMD3100)–mediated mobilization but not G-CSF–induced mobilization, (2) use of $SK1^{-/-}$ mice inhibits AMD3100 mobilization, (3) S1P plasma levels increase after AMD3100 treatment in the PB of mice but not humans, and (4) treatment of mice with a $S1P_1$ agonist (SEW2871) results in a dose-dependent augmentation of mobilization when co-administered with AMD3100. These data are consistent with other reports that suggest differing mechanisms of action between AMD3100 and G-CSF–induced mobilization of PBSCs. An unresolved question in the field is whether an increase in plasma S1P concentrations in the PB in response to stimulus is indeed a component of the mechanism by which PBSC mobilization occurs. Others have suggested that a S1P chemotactic gradient is continuously present in the PB, retention of HSCs/HPCs in BM is an active process that counteracts S1P gradient, and increased plasma S1P levels occurs during mobilization.[9] The data presented here confirm that that is true in mice but will have to be examined in humans. Additional data in humans as well as mice using highly sensitive assays that are able to detect subtle changes in relatively low concentrations of S1P may help to resolve this question. Even with this remaining controversy it is clear that a combination strategy to disrupt the endogenous HSC/HPC retention mechanisms, such as the SDF-1/CXCR4 axis, while augmenting the $S1P/S1P_1$ axis is likely to enhance PBSC mobilization and yield clinical benefit within the field of stem cell therapeutics/regenerative medicine.

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● ● ● IMMUNOBIOLOGY

Comment on Montecalvo et al, page 756

Functional transfer of microRNA by exosomes

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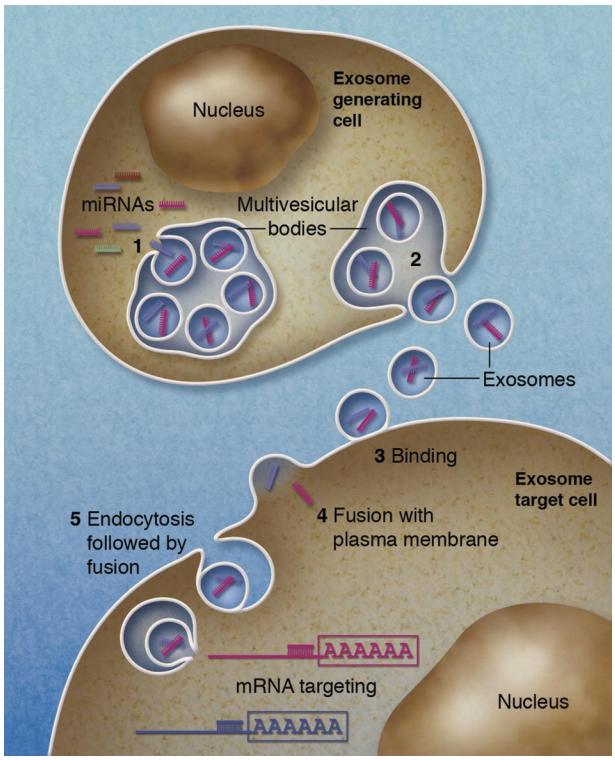
Cells can communicate directly with each other through cell-cell contact or at a distance using secreted soluble mediators. A third mode of intercellular communication may be mediated by exosomes, an emerging novel pathway with unprecedented potential. In this issue of *Blood*, Montecalvo and colleagues demonstrate that dendritic cells (DCs) secrete exosomes that are loaded with distinct sets of microRNA, dependent on the status of DC activation.[1] Moreover, they show that DC exosomes can fuse with target cells, thereby delivering their membranous and cytosolic contents. Finally, using a clever setup, they provide proof of principle that, after being transferred by exosomes, microRNA can repress mRNAs in target cells.



Schematic of microRNA transfer by exosomes. (1) microRNAs are selectively incorporated into the intraluminal vesicles of a multivesicular body. (2) Multivesicular bodies fuse with the plasma membrane, therewith secreting their intraluminal vesicles into the extracellular milieu. (3) Exosomes may bind to the plasma membrane of a target cell. Recruited exosomes may either fuse directly with the plasma membrane (4) or first be endocytosed and then fuse with the delimiting membrane of an endocytic compartment. (5) Both pathways result in the delivery of the exosomal microRNA to the cytosol of the target cell where it may associate with and silence corresponding mRNA. Professional illustration by Alice Y. Chen.

Cells may release membrane vesicles into their extracellular environment either by pinching them off directly from the plasma membrane or through secretion by endocytic compartments (reviewed in Théry et al[2]). Although many in vitro studies provide evidence that such released vesicles can be transferred to acceptor cells, confirmation of in vivo function(s) is still scarce. Extracellular vesicles have been assigned several names, including microvesicles and exosomes. The term exosomes is generally coined for those vesicles that are secreted as a consequence of the fusion of multivesicular bodies with the plasma membrane (see figure).

Multivesicular bodies are generated at endosomes by the inward budding of their delimiting membrane followed by the release of ~ 100 nm vesicles into the endosomal lumen. Such intra-endosomal vesicles have a cytosolic-side inward orientation, just like cells. Many multivesicular bodies serve as a sorting station for endocytosed membrane proteins that need to be transferred to and degraded in lysosomes. Other multivesicular bodies may instead fuse with the plasma membrane, resulting in secretion of their intraluminal vesicles as exosomes. Exosomes are secreted by many if not most cell types, and are abundantly present in body fluids such as blood, ascites, urine, milk, saliva, and seminal plasma. Our laboratory demonstrated that the secretion by DCs of MHC II carrying exosomes is specifically stimulated by MHCII-peptide interacting $CD4^+$ T cells,[3] suggesting that communication through exosomes is a regulated process. Indeed the protein content of DC exosomes varies with the status of maturation of the exosome producing DCs,[2] while DC exosomes can be targeted both to neighboring DCs or interacting T cells.[2,3]

Exosomes are proposed to have many distinct physiologic functions that may vary, depending on their cellular origin, from immune regulation, to blood coagulation, cell migration, cell differentiation, and other aspects of cell-to-cell communication. Exosomes have also been implicated in the pathogenesis of disease such as tumor development, cardiovascular disease, neurodegenerative disease and retroviral infection. This has sparked the idea that exosomes from body fluids may be useful as novel biomarkers for the detection and classification of disease, and perhaps can even be employed as therapeutic tools. This was first pioneered by Zitvogel and colleagues, who demonstrated over a decade ago that exosomes isolated from cultured DCs carried functional MHC-peptide complexes and could promote in vivo induction of antitumor immune responses in mice.[4]

Interest in exosomes was boosted further by a publication in 2007 from Valadi and colleagues demonstrating that exosomes from mast cells contain both mRNA and microRNA, and that at least some of these mRNAs could be translated into proteins on their transfer by exosomes to target cells.[5] Since then, exosomes from many other cell types have also been demonstrated to carry RNA, as summated in the database Exocarta.org. Multivesicular bodies are functionally linked to microRNA effector complexes,[6,7] perhaps indicating mechanisms for miRNA targeting to exosomes. Realization that exosomes may elicit epigenetic effects by transferring selected RNA molecules between cells has revolutionized our thinking of possible mechanisms of exosome signaling, and furthered ideas of using exosomal RNAs as biomarkers for disease.

The concept of exosome-mediated directed transfer of selected microRNA between cells is extremely attractive, although several basic elements of such a process required confirmation. Montecalvo and coworkers have now directly tested essential elements of this hypothesis by analyzing the mechanism of exosome-mediated transfer of microRNA between cells. Exosomes isolated from DC culture media enclosed > 200 microRNAs, with 5 uniquely detected in exosomes from immature DCs and 58 exclusively present in exosomes from mature DCs. These compositions differed from the microRNA content of the exosome-producing DCs, indicating selectivity for their incorporation into exosomes. Using a GFP-linked marker protein that was efficiently incorporated into exosomes, DC exosomes were demonstrated to be transferred to both bystander DCs and activated (but not naive) antigen-specific $CD4^+$ T cells. Exosomes were confirmed to fuse with target DC membranes in two independent assays, with one indicating membrane mixing and the other illustrating mixing of the exosomal luminal content with the cytosol of the target DC. Importantly, functional transfer of two exosomal microRNAs was demonstrated by

employing cells that were transfected with vectors encoding luciferase-coupled complementary targets.

Now that these in vitro requisites for transfer of exosome-shuttle microRNA have been demonstrated, a major future challenge will be to reveal physiologic relevance of this process. The molecular mechanisms that drive exosome formation and secretion have not yet been resolved. Although some Rab GTPases have been implicated, their depletion only partially interfered with exosome secretion (reviewed in Bobrie et al[8]). Dissection of the mechanism(s) that drive miRNA recruitment into exosomes may provide stronger tools to study the physiologic functions of miRNA transfer by exosomes.

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MYELOID NEOPLASIA

Comment on Wey et al, page 817

GRPling with PTEN

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Regulation of the Pten tumor suppressor is complex and mediated by varied mechanisms. In this issue of *Blood*, Wey and colleagues show in a biallelic conditional knockout mouse model of GRP78 and PTEN that heterozygous loss of *Grp78* suppresses leukemogenesis mediated by *Pten*.[1] These findings suggest a novel manner of down-regulation of PI 3 kinase signaling that may have potential therapeutic benefit.

PTEN (Phosphatase and tensin homolog on chromosome 10) is a major human tumor suppressor genes that is frequently inactivated through mutation, deletion, or promoter methylation in tumors such as glioblastoma, endometrial, breast, thyroid, and prostate cancers (reviewed in Cully et al,[2] and Martelli et al[3]). In addition, germ line mutations of *PTEN* lead to PTEN hamartoma tumor syndrome, a cancer predisposition condition. PTEN is a lipid phosphatase that has catalytic activity on the D-3 phosphate of the active lipid second messenger phosphatidylinositol 3,4,5-triphosphate (PIP_3).[4]

PTEN mRNA expression is regulated by promoter hypermethylation, a diverse range of transcriptional control and miRNA.[3] All phosphatase enzymes possess a cysteine nucleophile at the catalytic site that is subject to oxidation. Serine and threonine phosphorylation of PTEN at the carboxy terminus locks PTEN in a stable conformation that reduces membrane localization and enzymatic activity. PTEN is also nuclear localized and the function and stability of PTEN in different, unique cellular environments has not been extensively investigated.

Gene-targeting experiments revealed that $Pten^{-/-}$ mice had defective endodermal, ectodermal, and mesodermal differentiation,[5,6] suggesting that *Pten* is required for mouse embryogenesis. Heterozygous animals developed germ cell, gonadostromal, thyroid, and colon tumors. Murine embryo fibroblasts isolated from $Pten^{-/-}$ mice showed enhanced Akt phosphorylation, suggesting that Pten is a negative regulator of PI 3 kinase signaling.[7] Conditional inactivation of *Pten* in the hematopoietic lineage results in a myeloproliferative disease followed by onset of leukemia with a latency of 4 to 6 weeks.[8]

GRP78 (Glucose-regulated Protein of 78 kDa) is a member of the HSP70 (Heat-shock protein 70) gene family, and is thought to be an endoplasmic reticulum (ER) chaperone and a marker of ER stress (reviewed in Ni et al[9]). Recent studies suggest GRP78 may be found outside the ER, especially in transformed cancer cell lines. GRP78 can be found secreted, at the membrane, in the cytosol, within mitochondria or in the nucleus. α_2-macroglobulin is proposed to bind GRP78 and couple to PI 3 kinase pathway activation in tumor cells. Therefore, the possibility exists that GRP78 could regulate members of the PI 3 kinase pathway, including PTEN.

Wey et al initially tested this hypothesis by performing compound crosses using conditional alleles of *Grp78* and *Pten* crossed with a probasin-Cre reporter.[10] Loss of *Grp78* did not affect the development of the prostatic epithelium. However, homozygous deletion of *Grp78* in a *Pten*-deficient background had a profound effect on prostate adenocarcinoma development. Importantly, $Pten^{fl/fl}Grp78^{fl/fl}$ mice displayed absent phosphorylation of Akt whereas $Pten^{fl/fl}Grp78^{+/+}$ mice had robust phospho-Akt staining in dorsolateral prostate sections.

Wey et al extended these studies. They showed that biallelic targeting of $Pten^{fl/fl}$ and $Grp78^{fl/+}$ in an Mx1-Cre background resulted in an increase in disease latency from 4 weeks in $Pten^{fl/fl}$ animals to 7 weeks in $Pten^{fl/fl}Grp78^{fl/+}$ mice. Heterozygous loss of *Grp78* suppressed blast cell formation in the bone marrow, whereas spleen weight and percent of $Lin^-Sca1^+Kit^+$ cells were intermediate in $Pten^{fl/fl}Grp78^{fl/+}$ comparing $Pten^{fl/fl}$ and wild-type mice.

Phosphorylation of Akt and the downstream target of Akt signaling, the S6 kinase, were increased in $Pten^{fl/fl}$ bone marrow cells. Loss of one copy of *Grp78* resulted in suppression of Akt and S6 kinase phosphorylation in $Pten^{fl/fl}Grp78^{fl/+}$ bone marrow. These data were complemented by siRNA experiments that showed knockdown of GRP78 results in decreased phosphorylation of AKT in the HL60 cell line. Wey and colleagues also performed in vitro studies demonstrating that