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Brief Discussion on Metastatic Bone Osteolysis

Pérez Jessica*

Department of Medicine, McGill University, Canada

Abstract

Bone-resorbing osteoclast activation facilitates breast and prostate carcinoma bone metastasis. We have identified peroxiredoxin-4 (PRDX4) as a mediator of osteoclast genesis that is released by cancer cells through proteomics methods. Using immunoblotting and mass spectrometry, we now report the characterization of L-plastin in MDA-MB-231 human breast cancer cells' conditioned media (CM). With L-plastin silenced by siRNA, the osteoclastogenic potential of MDA-MB-231 CM was significantly diminished. L-plastin was found in cancer-derived exosomes, and MDA-MB-231 CM's osteoclastogenic capacity was significantly diminished when exosomal release was inhibited. Recombinant L-plastin induced calcium/NFATc1-mediated osteoclastogenesis to levels comparable to continuous RANKL treatment when added to osteoclast precursors primed with RANKL for two days. We used ssRNA to generate MDA-MB-231 cells devoid of PRDX4, L-plastin, or both, and injected these cell populations into CD-1 immunodeficient mice via the tibia. When MDA-MB-231 cells lacking both L-plastin and PRDX4 were injected, micro-CT and histomorphometric analysis revealed a complete loss of osteolysis. Multiple human cancers, including breast and prostate carcinomas, showed an increase in L-plastin and PRDX4 mRNA expression, according to a meta-analysis. This study demonstrates that human breast cancer cells activate osteoclasts through the secretion of L-plastin and PRDX4.

Keywords: Rankle, L-plastin; Osteoclasts; Osteoclastogenic

Multiple tumor metastases, including breast, prostate, lung, and kidney carcinomas, commonly spread to bone. Both osteolytic and osteoplastic bone metastases are facilitated by osteoclasts, bone cells that specialize in bone destruction. Monocytic precursors of hematopoietic origin fuse to form osteoclasts. Osteoclast formation is triggered by the nuclear factor kB receptor activator (RANK) and its ligand (RANKL). The osteoblast-specific transcription factor nuclear factor of activated T cells (NFAT) c1 is expressed and activated through RANK signaling, which also includes the activation of the transcription factors NF-kB and AP-1 and the protein kinases JNK, ERK, and p38. In the cytosol, hyper phosphorylated NFATc1 remains inactive [1]. NFATc1's nuclear translocation is made possible by calcineurin, a phosphatase that dephosphorylates NFATc1 and is stimulated by calcium signaling, particularly by the calcium oscillations that are observed in osteoclast precursors. For the stimulation of calcium/NFATc1 signaling and osteoclastogenesis, co-stimulatory immunoglobulin-like receptor signals, such as the osteoclast-associated receptor (OSCAR) and the triggering receptor expressed in myeloid cells-2 (TREM-2) are also essential [2].

While Rankle is required in the early stages of osteoclastogenesis, we previously demonstrated that factors produced by actively proliferating breast or prostate cancer cells that activated the ERK and calcium/NFATc1 signaling pathways in osteoclast precursors can stimulate osteoclast formation from late precursors independently of RANKL. We confirmed that removal of IgG-bound factors from media conditioned by MDA-MB-231 breast carcinoma cells significantly reduced its osteoclastogenic potential and identified one of the factors as peroxiredoxin-4 (PRDX4). Since co-stimulatory immunoglobulin-like receptors are essential for inducing calcium oscillations and sustained NFATc1 activation [3], we investigated whether the relevant mediators could be immunoprecipitated with IgG. In this study, we identify L-plastin as an additional osteoclastogenic factor released by breast cancer cells and investigate how PRDX4 and L-plastin work together to stimulate osteolysis in an experimental bone metastasis model of breast cancer.

L-plastin is one of three mammalian isoforms in a family of actin-binding proteins that share 75% to 80% homology: Small intestine, colon, and kidney expression of I-plastin (plastin-1, fimbrin); L-plastin, also known as plastin-2 or LCP1, is mostly found in hematopoietic cells; and the presence of T-plastin (plastin-3, T-fimbrin) in solid tissues. Cross-linking of actin filaments has been linked to all plastins [4]. L-plastin is primarily expressed physiologically in hematopoietic cells; However, L-plastin is expressed by a variety of non-hemapoietic malignant human cells. L-plastin has not yet been shown to have any extracellular function; however, extracellular fluids have been found to contain it. We found that MDA-MB-231 human breast cancer cells secrete a novel soluble factor called L-plastin in this study. We investigated the mechanism by which L-plastin stimulates osteoclastogenesis and the route by which it is secreted. The contribution of L-plastin and PRDX4 to cancer-induced osteolysis was evaluated using an in vivo model of experimental bone metastases. Finally, publicly accessible datasets of differential gene expression in cancer patients confirmed the significance of L-plastin and PRDX4 as a diagnostic and prognostic factor for the progression of various types of cancer [5].

Result

Six-week-old C57BL6/J mice (Charles River) served as the source of the mouse bone marrow cells. Nonadherent cells were collected and plated at 5 x 10 4 cells/cm 2 in -MEM medium supplemented with 100 U/ml penicillin, 100 g/ml streptomycin, and 10% fetal bovine serum, M-CSF (50 ng/ml), and recombinant GST-RANKL (100 ng/ml) for 24 hours. Every two days, the medium was changed out. On day 5, the cell cultures were stained for tartrate-resistant acid phosphatase (TRAP, Sigma-Aldrich, and 387A-KT) and fixed with 10% formalin. RAW 264.7 cells (TIB-71, American Type Culture Collection) were cultured in DMEM supplemented with L-glutamine, 1 mM pyruvate, 100 units/

***Corresponding author:** Pérez Jessica, Department of Medicine, McGill University, Canada, E-mail: jper@usal.es

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ml penicillin, 100 g/ml streptomycin, and 10% FBS, and osteoclasts were identified as multinucleated (cells with more than three nuclei) TRAP-positive cells. Recombinant GST-RANKL (50 ng/ml) was added 24 hours after RAW 264.7 cells were plated at 5 10 3 cells/cm 2. After being washed, treated with recombinant L-plastin (rP2, 2.5-25 g/ml), cultured for two days, fixed, and stained for TRAP, cells were added to fresh media on days 2-3 with or without. who works at the University of Ghent in Belgium, provided the L-plastin [6].

After loading samples containing 50 g of proteins onto a 10% SDS-PAGE, staining and destaining were performed. After being removed from the gel, the desired band was digested with trypsin, alkylated with iodoacetic acid, and reduced with DTT [7]. A Thermo Acclaim Pepmap (Thermo, 75 M ID 2 cm C18 3-M beads) precolumn and an Acclaim Pepmap Easyspray (Thermo, 75 M ID 15 cm with 2-M C18 beads) analytical column were used to separate the lyophilized peptides over the course of two hours using a Dionex Ultimate 3000 HPLC at 220 nl/min with an organic gradient of A Thermo Orbitrap Fusion mass spectrometer with 120,000 resolutions (FWHM in MS1, 15,000 for MS/MS) and HCD sequencing of all peptides with a charge of 2+ or higher was used to analyze the peptides. Mascot 2.3 was used to perform a search against human sequences using the converted raw data in the *.mgf format (Mascot generic format). Scaffold Q+ Scaffold_4.4.8 (Proteome Sciences) was used to load the results of the database search for spectral counting, statistical analysis, and data visualization.

Discussion

We previously demonstrated that peroxiredoxin-4 (PRDX4) is one of the mediators of these osteoclastogenic effects and that soluble factors produced by breast and prostate cancer cells induce osteoclast differentiation. Plastin was another protein that was found in the osteoclastogenic fraction by mass spectrometry. It is common practice to refer to the three plastin isoforms I-plastin (PLS1), L-plastin (LCP1), and T-plastin (PLS3) as intracellular proteins. We first confirmed the antibodies' specificity for various plastin isoforms due to the high degree of homology between the isoforms. The I-plastin antibody exhibited high nonspecific binding at lower molecular weights, whereas the T- and L-plastin antibodies were specific; A product was found in both T- and I-plastin lysates by an antibody against T-plastin; The molecular weight, on the other hand, was lower than expected. Using immunoblotting, we next investigated the protein levels of plastin isoforms in MDA-MB-231 cell lysates and conditioned media (CM). The lysates of breast cancer cells contained each and every plastin isoform; however, in the MDA-MB-231 CM, only L-plastin was found extracellularly. Using mass spectrometry, we analyzed the electrophoresis band between 60 and 70 kDa. We found 13 peptides that came from L-plastin and 5 peptides that came from T-plastin, with a 95% probability. The ability of MDA-MB-231 cells to induce osteoclastogenesis was decreased following a brief siRNA knockdown of L-plastin. In a similar manner, the ability of mouse breast carcinoma 4T1 cells to stimulate osteoclast formation was diminished when L-plastin was knocked out. In breast, prostate (PC3), and erythroleukemia (K562) cancer cells, L-plastin was found in cell lysates, but not in normal skin fibroblasts (NSF). Intriguingly, we frequently observed two bands in the majority of CM samples and some cell lysates, pointing to the possibility of splice variants or proteolytic products. So, we show that breast cancer cells express and secrete L-plastin, which may be involved in stimulating osteoclastogenesis.

L-plastin (current manuscript) and PRDX4 (13), two established cytosolic proteins, have been shown to be actively released from breast cancer cells and to participate in intercellular communications. L-plastin was only reported to function as an actin binding protein involved in motility and invasion, whereas PRDX4 had a signal peptide that allowed for its secretion and was found in the extracellular space. We assumed that the cancer secretome consists of macromolecules released from cancer cells by all means, including secretion, shedding, and vesicular release, in order to evaluate the method by which L-plastin is released from breast cancer cells. Exosomes released by cancer cells have been found to contain numerous cytosolic proteins. Because inhibition of exosomal release significantly reduced the osteoclastogenic potential of breast cancer-derived CM, we discovered that exosomes were necessary for the formation of osteoclasts in response to breast cancer.

Conclusion

L-plastin was found in the exosomal fraction of media conditioned by breast cancer cells, whereas PRDX4 was not. This was demonstrated by purifying exosomes from the media. Exosome-depleted CM also contained detectable levels of L-plastin, indicating either the spillage of L-plastin from exosomes or the existence of a different pathway for its release. In this regard, it has been demonstrated that exosomes communicate with the cells they are targeting through a variety of channels, such as the following: i) interaction of a ligand on the outer surface of the exosomal membrane with a cell surface receptor; ii) Exosomal membrane proteolysis results in the release of soluble ligands; or iii) after being taken up by the target cells, the exosomal content is released into the cytoplasm. Our findings indicate that extracellular L-plastin contributes to cancer metastasis to bone by fostering a favorable osteolytic microenvironment, in addition to the established prometastatic function of intracellular L-plastin, which is known to increase cancer invasiveness.

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