Annexin A2 Expression in Prostate Cancer Cells.

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ABSTRACT

Background: Metastasis is a major cause of morbidity in prostate cancer patients, the primary mortality in this disease is metastasis to the bone tissue. Despite substantial efforts to understand prostate cancer metastasis, the mechanisms that are involved in preparing the metastatic niche for colonizing the prostate cancer cells are still not known. Therefore, there is an urgent need to identify essential regulators of bone metastasis in prostate cancer for therapeutic targets.

Purpose: Annexin A2 (AnxA2), a calcium-dependent phospholipid binding protein, is overexpressed in the poorly differentiated high-grade adenocarcinomas of prostate cancer. AnxA2 exists as a monomer in the cytosol and as a heterotetrameric complex with S100A10 [(AnxA2)2-(S100A10)2] at the cell surface. Phosphorylation of AnxA2 at tyrosine 23 (pAnxA2-Y23) is an important event for the localization of AnxA2 to the cell surface. At the cell surface, AnxA2 heterotetramer complex provides binding site for tissue plasminogen activator (tPA) and converts plasminogen into plasmin, which plays an important role in invasion and metastasis of cancer. The cell surface AnxA2 also plays an important role in hematopoietic stem cell localization to the marrow niche and regulates osteogenic differentiation. However, the cell surface expression of AnxA2 in prostate cancer is unknown. Therefore, in the present study, we have demonstrated the cell surface expression of AnxA2 in prostate cancer cells to delineate the mechanism of bone metastasis.

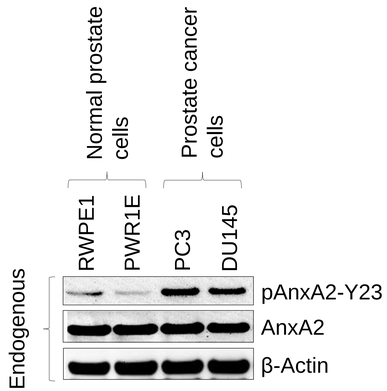
Methods: Prostate cancer cell lines, PC3 and DU145 were grown in RPMI-1640 medium containing 10% fetal bovine serum, in a humidified incubator at 37°C with 5% CO_2. The RWPE1, and PWR-1E cells were cultured in keratinocyte growth medium supplemented with 5 ng/ml human recombinant epidermal growth factor and 0.05 mg/ml bovine pituitary extract (Invitrogen, Carlsbad, CA) and maintained in an incubator under the conditions described above. Immunoblotting was used to detect the expression of pAnxA2-Y23 and AnxA2 proteins in cells.

Results: Our results demonstrated that the expression of pAnxA2-Y23 is very high in prostate cancer cells (PC3 and DU145 cells) compared to normal prostate epithelial (PWR1E, and RWPE1 cells). However, the expression of total AnxA2 in both prostate normal and cancer cell lines is comparable. In addition, our membrane wash experiment showed that a large amount of AnxA2 is present at the cell surface of the PC3 and DU145 cell lines. In normal prostate epithelial cells, even though the expression of total AnxA2 is comparable to PC3 and DU145 prostate cancer cells, membrane localization of AnxA2 is very low.

Conclusion: Our results clearly suggest that the cell surface expression of AnxA2 is high in prostate cancer cells due to increased phosphorylation of AnxA2 at tyrosine 23.

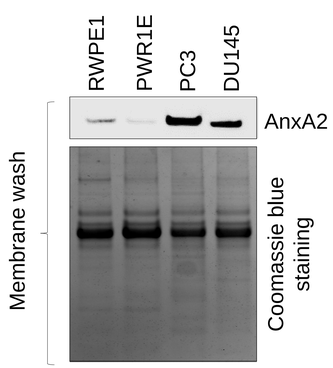
RESULTS

Expression of pAnxA2-Y23 and AnxA2 in human prostate normal and cancer cell lines:



The expression of pAnxA2-Y23 and total AnxA2 were analyzed by immunoblotting in prostate normal and cancer cell lines. Our results demonstrated that expression of pAnxA2-Y23 is high in prostate cancer cell lines (PC3 and DU145 cells) compared to prostate normal (PWR1E, and RWPE1) cell lines. However, the expression of total AnxA2 in these cell lines is comparable. The expression of β-actin was used as loading control.

Cell surface expression of AnxA2 in human prostate normal and cancer cell lines by membrane wash experiment:



Increased phosphorylation of AnxA2 at Y23 is associated with increased translocation of AnxA2 to the outer surface of the cells. Therefore, we compared the cell-surface localization of AnxA2 in prostate normal and cancer cell lines. AnxA2 heterotetramers are Ca^{++}-dependent phospholipid-binding proteins, and are stripped from the cell surface by Versene (0.53 mM EDTA in PBS). Therefore, cells were incubated with Versene for 5 min and then supernatant was collected by centrifugation for immunoblotting. Our results demonstrated that in PC3 and DU145 cell lines, a large amount of AnxA2 is present in the membrane wash fraction compared to normal prostate epithelial (RWPE1 and PWR1E cells) cell lines. Coomassie blue staining of Versene eluates was used as a loading control.

IMPORTANT FINDINGS

- AnxA2 phosphorylation at tyrosine 23 is significantly high in prostate cancer cells compared to normal prostate epithelial cells.
- The cell surface expression of AnxA2 is significantly high in prostate cancer compared to normal prostate epithelial cells.

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