Role of ASH1L in Prostate Cancer Metastasis

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Background

ASH1L (Absent, Small, or Homeotic 1-Like) is a histone lysine methyltransferase (KMT) that catalyzes the methylation of histone 3 on lysine 36 (H3K36) [1]. ASH1L methylates the H3K36me2 mark specifically, which regulates gene expression in leukemia cells. The catalytic SET domain is responsible for its regulatory actions. ASH1L has been known to play a role in acute leukemia progression and poor prognosis [2]. The overexpression of ASH1L was found in breast, liver, and thyroid cancer and has been linked with increased cancer cell growth and disease aggression [3, 4, 5]. However, the activity of ASH1L has not been explored in prostate cancer (PCa) metastasis, as this study attempts to do. Since the 5-year survival rate for PCa drops from 100% to 30% with the development of metastasis, exploring this mechanism is vital to decreasing PCa mortality [6].

Methods

Data for bioinformatics analysis was gained from The Cancer Genome Atlas prostate cancer patient samples.
Cell migration and 3D invasion assays were performed on PC3M control and ASH1L knockdown cells. Analysis of invasion assay was conducted with NIH ImageJ software. Maximum invasion length was found by subtracting the original radius of the cell from furthest invasion point and invasion area was found by subtracting the total area of invasion by the cell area.
Real-time PCR (qPCR) was performed using SYBR Green reagent (Applied Biosystems) in LNCap control and ASH1L overexpressed cells.
Western blot was performed to determine protein expressions of methylation of H3 marks and EMT transcription factors.
Immunofluorescence (IF) was performed with in bone sections derived from DX1 intratibial injected mice and detected on a VectraPolaris scanner (PerkinElmer).

Results

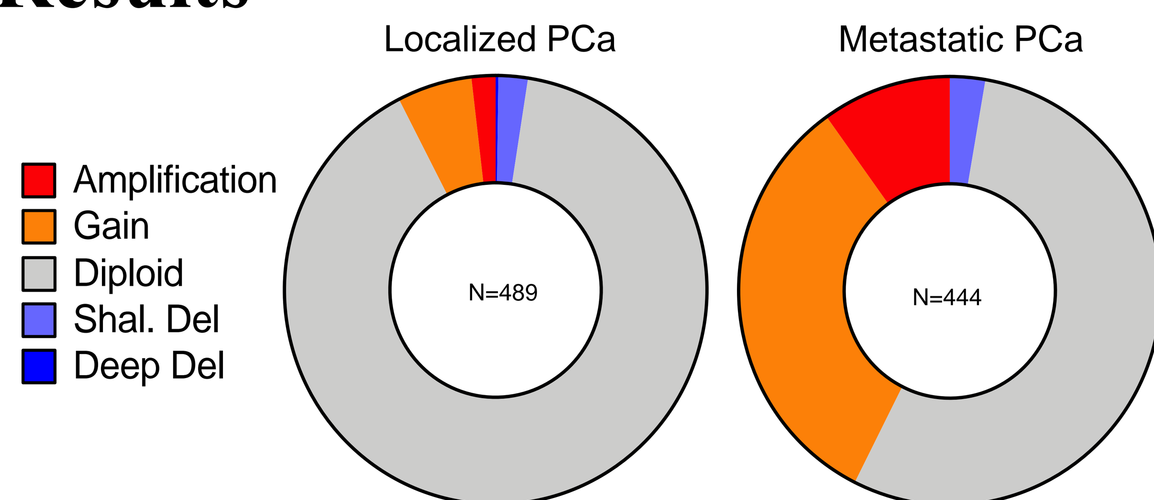

Figure 1. *Genetic alterations of ASH1L in human PCa samples.* Bioinformatics analysis of five different ASH1L genetic alterations in localized (left) and metastatic (right) PCa samples from The Cancer Genome Atlas (TCGA) database.

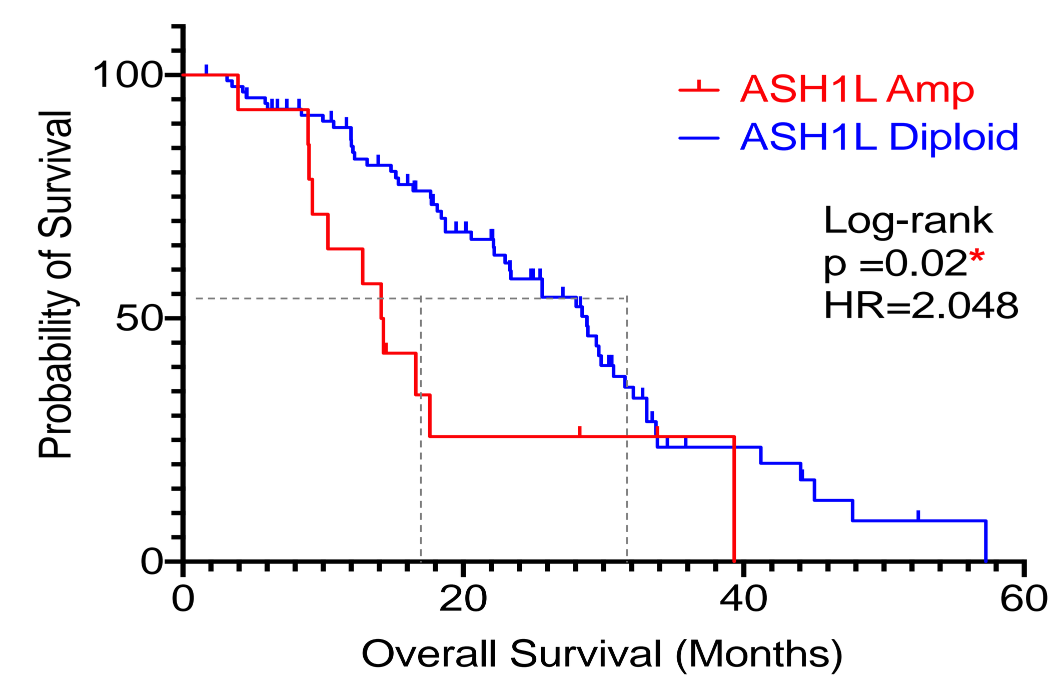

Figure 2. *Overall survival plots of ASH1L amplified and diploid patients.* Patients with ASH1L amplification had a probability of Survival and overall survival that dropped significantly when compared with normal ASH1L expression. Gray dashed lines indicate half-life of patients or 50% of survival. *p* value was calculated by log rank test. *p < 0.05.

Results

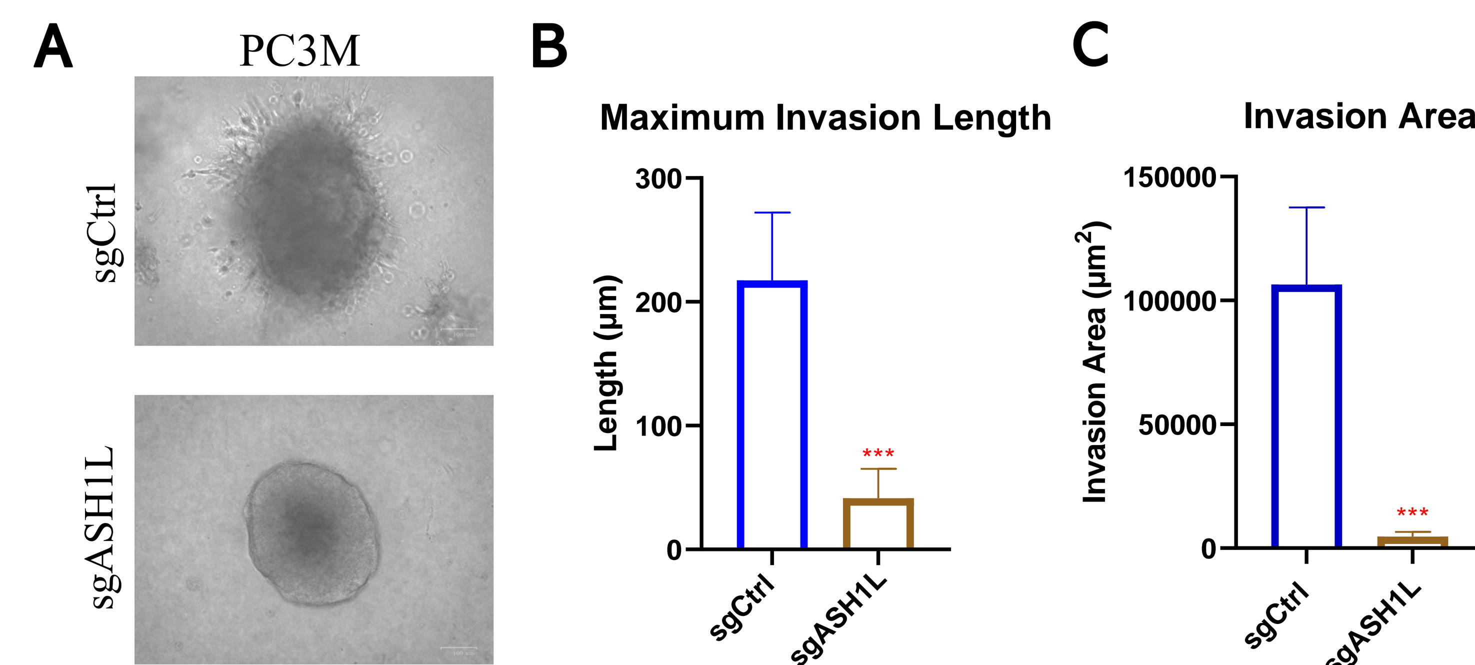

Figure 3. *3D Matrigel drop invasion assay in PC3M control and ASH1L knockdown. (A)* Representative images of the control and ASH1L knockdown group. Images of the cells were taken 72 hours after seeding and analyzed for invasion and general morphology changes.
(B) and (C) Quantification of invasion capacity with maximum invasion length and invasion area in the control and ASH1L knockout group. *p* values were calculated by by two-tailed Student's t test. ***p < 0.001.

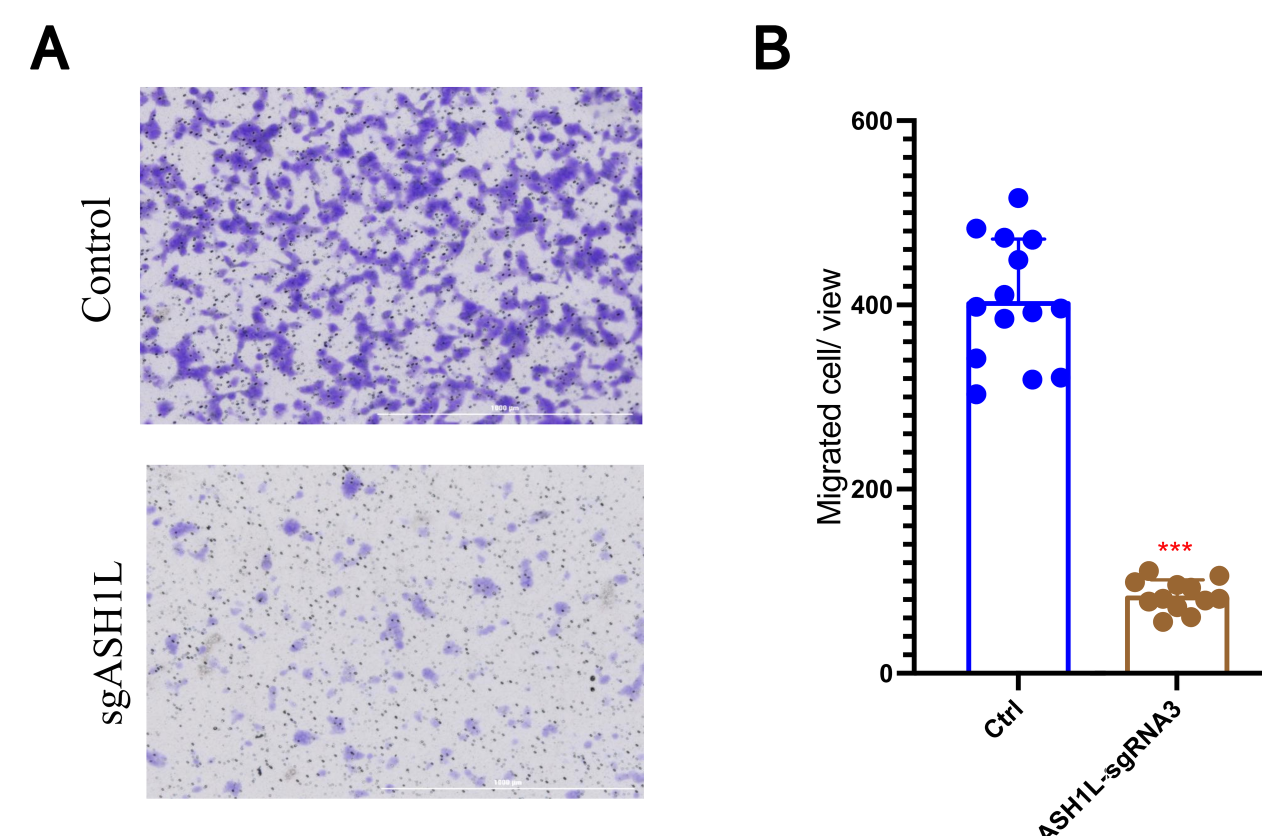

Figure 4. *Migration assay in PC3M control and ASH1L knockdown.*
(A) Representative images of the control and ASH1L knockdown groups in PC3M. Images of the cells were taken 48 hours after seeding in the inserts.
(B) Quantification of cells per image section from migration assay in PC3M control and ASH1L knockdown. *p* values were calculated by by two-tailed Student's t test. ***p < 0.001.

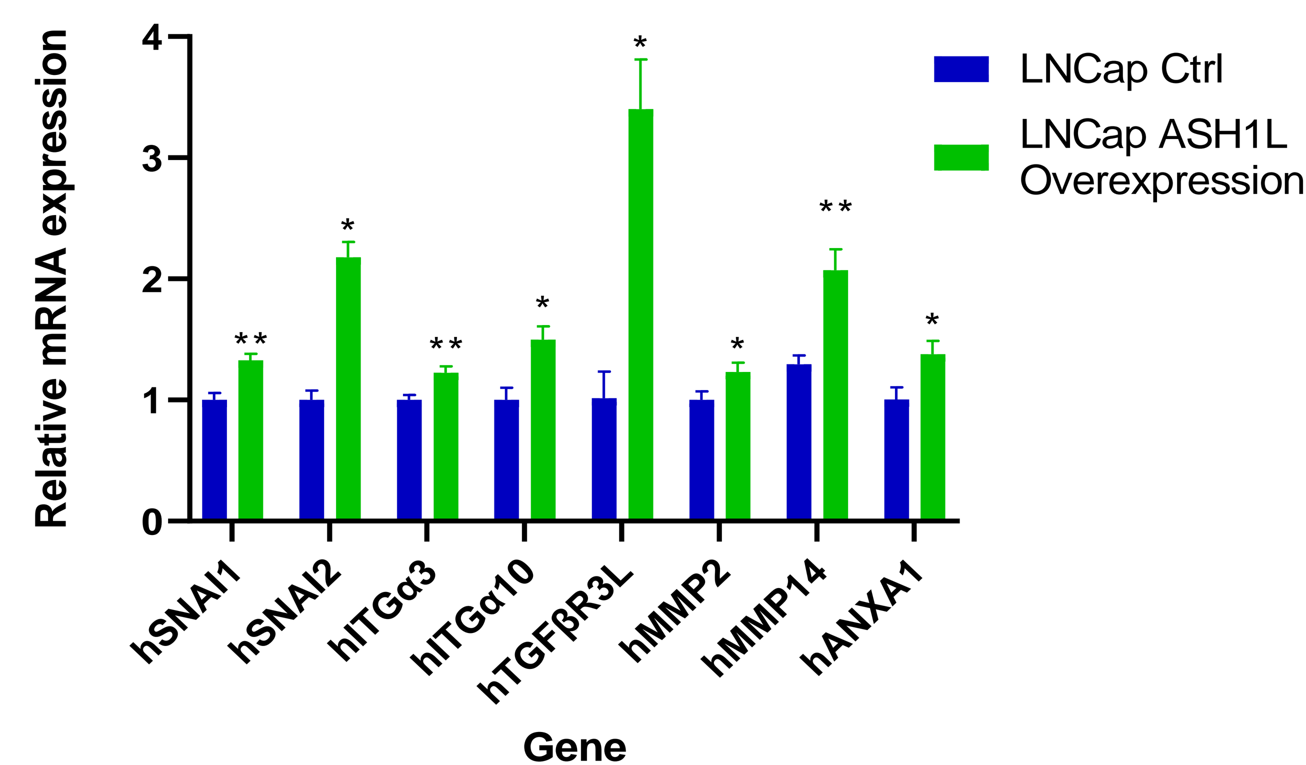

Figure 5. *Relative mRNA expression of pro-metastatic genes in LNCap control and ASH1L overexpression.* SNAI1 and SNAI2, implicated in cancer cell invasion, were both upregulated, as well as several matrix metalloproteinase (MMP) family genes, shown to relate to poor prognosis and invasion [7, 8]. ITGA3 and ITGA10 are also involved in tumorigenesis and tumor proliferation, along with TGFβ and ANXA1 [9, 10, 11, 12]. All the proteins these genes encode have been linked to the epithelial-mesenchymal transition (EMT), which gives cancer cells metastatic properties to invade peripheral tissue [13]. *p* values were calculated by by two-tailed Student's t test. *p < 0.01. **p < 0.01.

Results

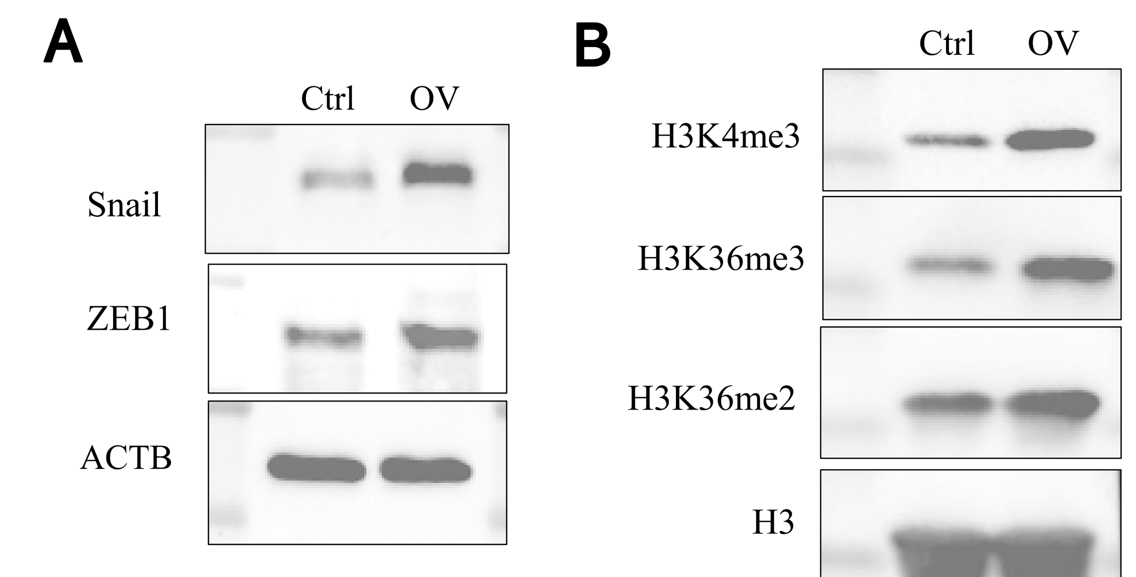

Figure 3. *Western blot for ASH1L overexpression (OV) in LNCap cells.*
(A) Protein expressions for epithelial–mesenchymal transition (EMT) transcription factors: zinc finger E-box binding homeobox 1 (ZEB1) and Snail family transcription factor 1 (SNAI1)
(B) Protein expressions for methylation of histone H3 markers which have been reported to be regulated by ASH1L.

Conclusions and Future Directions

The findings of this project support the contribution of ASH1L overexpression to human PCa metastasis. Gain of function of ASH1L is highly correlated with metastases in human PCa samples, as well as the in vitro invasion assay yielded significant limitations for invasion capacity in ASH1L KO cells. ASH1L overexpression results in upregulation of EMT transcriptional factors, which regulate cell migration and metastasis.
Additionally, the mechanisms of ASH1L are further being studied in vivo with promising mouse models. We performed intratibial injection (I. T) with DX1 cells in B6 mice to mimic bone formation caused by prostate cancer in human. The effects of ASH1L overexpression and KO on the tumor microenvironment are also being explored in vivo. Here, IF results shown in Figure 6, tumor associated macrophages were accumulated in bone formation regions compared to normal regular bone region. And importantly, development of an inhibitory compound for ASH1L could potentially prevent metastasis from forming in a patient diagnosed with primary PCa.

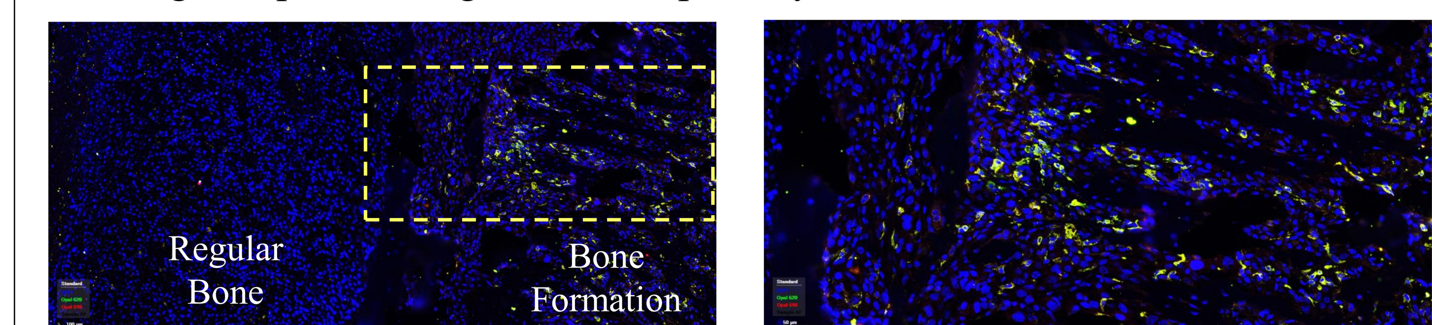

Figure 6. *Immunofluorescence on DX1-derived syngeneic bone metastatic model showing macrophage infiltration.* CD206 (green) and CD68 (red) indicated cell surface markers for M2 and total macrophage, respectively. 10x magnification is shown on the left and 20x magnification is shown on the right.

Resources

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