A Zebrafish Model of Osteosarcoma Metastasis Identifies Versican (VCAN) and Extracellular Matrix Remodeling Pathways as Drivers of Migration and Extravasation

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Background

- Osteosarcoma (OS) is the most common primary bone cancer in humans.[1]
- The 5-year overall survival for OS is dismal and no new effective therapies have been discovered since the 1980s.[2,3]
- A major challenge to discovery of new treatments is in having reliable models to study therapeutic vulnerabilities for metastatic OS.
- This study utilizes a novel Zebrafish model to identify specific cells that are able to extravasate out of the vasculature, obtain them, and grow them in culture[4] and identify targets that may be helpful in future treatments of metastatic cancers.

Purpose

- For metastatic OS patients, it is critical to identify molecular mechanisms and pinpoint new therapeutic interventions to prevent metastatic spread.

METHODS

CELL CULTURE
- 3 OS Cell Lines: two canine – HM-POS[5] and D17; one human- 143B
- Fluorescently-labeled cells were injected into the vasculature of zebrafish larvae (48 hours post fertilization; **Figure 1**)
- Isolated extravasated cells were collected and grown in culture for one week prior to RNA-Seq
- Pathway enrichments were inferred using gene set enrichment analysis using Hallmark[6] and Reactome[7] databases.

VCAN KNOCKDOWN AND MIGRATION ASSAYS
- Four independent VCAN siRNAs (Qiagen, Venlo, Netherlands) were used; VCAN knockdown was verified by qRT-PCR; scratch wound assays were quantified in ImageJ.

ANALYSIS OF VCAN AS A BIOMARKER OF CLINICAL RESPONSE
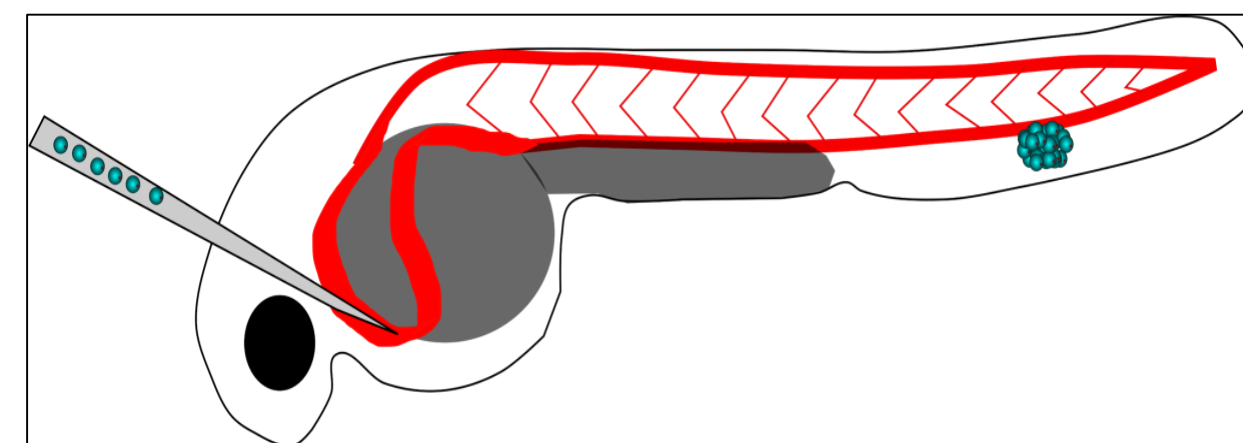- The prognostic significance of VCAN was determined utilizing R2 Genomics.

Figure 1: Dog osteosarcoma cells being injected into our zebrafish model. Extravasated cells were collected and grown in culture.

Results

PATHWAY-BASED ANALYSIS SHOWS SUBSTANTIAL OVERLAP ACROSS METASTASIS MODELS
- D17 and HMP-OS share 15 upregulated genes and approximately eight (8) downregulated pathways (**Figure 2A**).
- Genes at the pathway level were then arranged by their normalized enrichment score (ES) with purple indicating upregulation of the gene and green indicating down regulation of the gene (**Figure 2B**).
- Two pathways of interest emerged (**Figure 2C, 2D**):
  - Upregulation of the E2F pathway
    - Upregulation of cell-division cycle protein 25C (CDC25C) and centrosome-associated protein E (CENPE)
    - Downregulation of cell-division cycle protein 20 (CDC20)
  - Downregulation of the extracellular matrix
    - Upregulation of VCAN
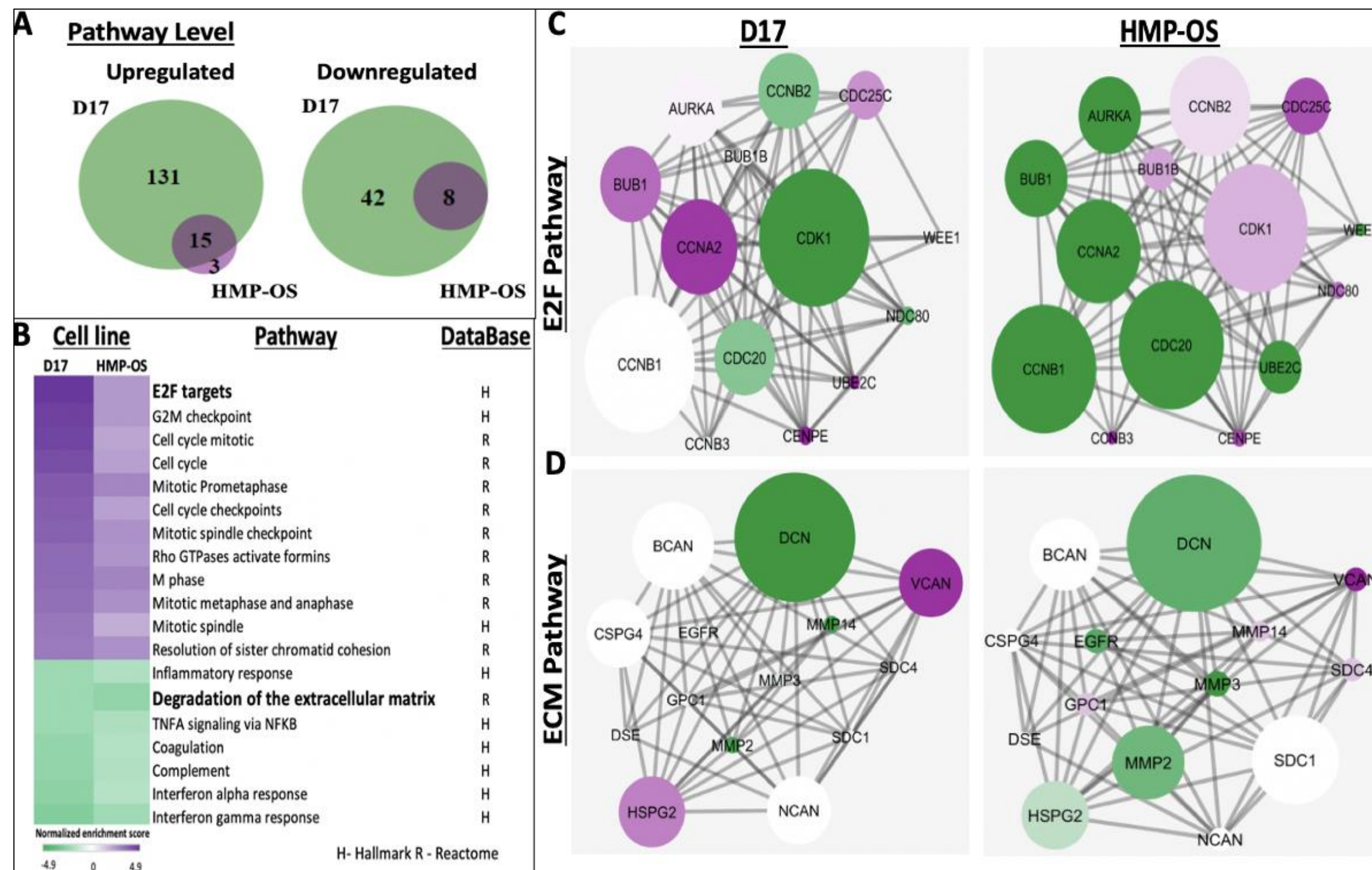   - Downregulation matrix metallopeptidase 2 (MMP2)

Figure 2: A) Overlap of upregulated and downregulated genes between HMP-OS and D17 osteosarcoma cell lines. B) GSEA showing an increased enrichment score in both D17 and HMP-OS for E2F targets and a decreased enrichment score for degradation of the extracellular matrix. C) Pathways of genes that encompass the E2F targeting pathway. The green circles represent genes that are downregulated and the purple represents genes that are upregulated. Both cell lines have upregulated CDC25C and CENPE and downregulated CDC20 in the E2F pathway. D) The ECM degradation pathway with green circles representing genes that are downregulated and purple circles representing genes that are upregulated. Both cell lines have upregulated VCAN and downregulated MMP2.

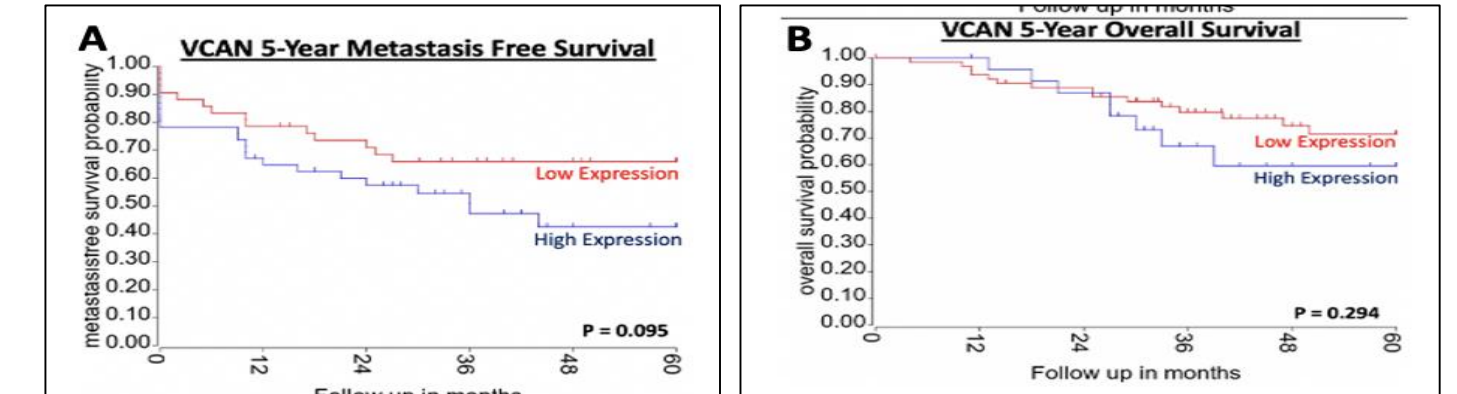### PROGNOSTIC SIGNIFICANCE OF VCAN IN-VIVO

Figure 3: A) VCAN's 5-year metastasis free survival had a p-value of 0.095 for the low expression group versus the high expression group. B) The 5-year overall survival between the high and the low expression groups for VCAN was found to have p-value of 0.294.

QPCR AND SCRATCH WOUND
- Consistent with a role in invasion/metastasis, knockdown of VCAN (Figure 4A) significantly inhibited OS cell migration (Figure 4B,C) in human 143B cells.
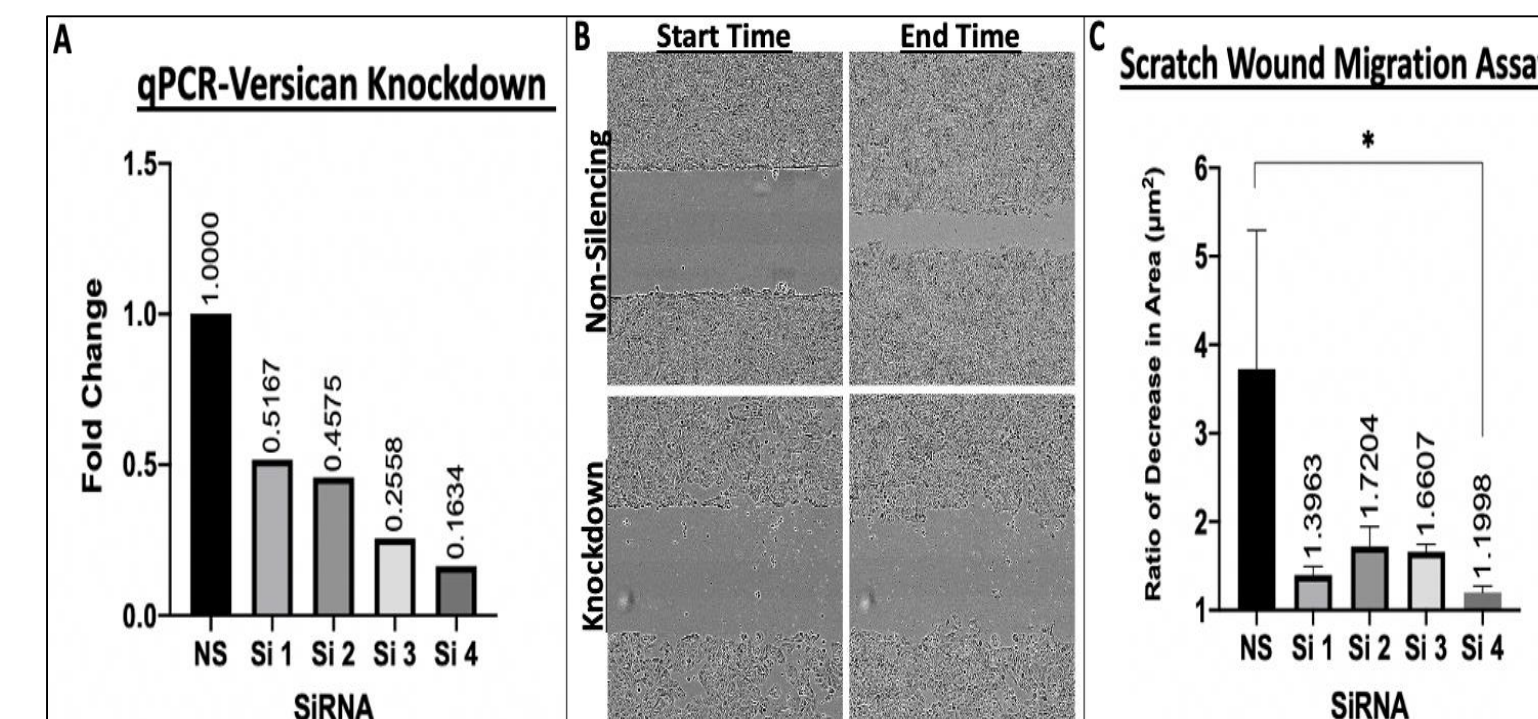

Figure 4: A) qPCR demonstrating VCAN knockdown in 143B osteosarcoma cells. B) Photo of scratch wound assay providing a visual representation of the difference in migration between the control condition (non-silencing) and the knockdown condition. C) Scratch wound migration assay showing that all siRNAs inhibited migration of cells when compared to the control condition.

Discussion

- Despite having no prognostic significance from the R2 *in-vivo* data VCAN was chosen as the ECM was of particular interest to this group and the results may be related to the sample in the R2 group:
  - Mostly Osteoblastic osteosarcoma (least metastatic potential)[8]
  - Small sample size
- VCAN may be a target for future drug therapies to combat metastatic osteosarcoma.
- Based on the model created by Allen and colleagues (2017) we created a model for hematogenous spreading osteosarcomas that was able to identify genes that might be important to target and, theoretically, could be applied to other hematogenously, metastasizing tumors.

References

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