Pathway Analysis of Major Genes Affecting Osteosarcoma Metastasis

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Introduction

Background

Osteosarcoma (OS)

- Most common primary malignant tumor of bone in humans and dogs.
- Rare (5th most common malignancy in children and adolescents) and commonly occurs in the metaphyseal area of long bones such as the proximal humerus, distal radius, distal femur, or proximal tibia.
- Overall survival rate is 65-70% in patients presenting without detectable metastases, survival rate plummets in patients who have macroscopic metastases at diagnosis.
- Prior work in both dogs and humans demonstrated that nearly all patients have micro-metastases present in the lungs at diagnosis. In a subsection of these patients, standard chemotherapy and surgery is insufficient to prevent development of chemoresistant macrometastases.

Hyaluronic Acid (Hyaluronan, HA)

- Major structural component of the extracellular matrix (ECM), found specifically in the extracellular, pericellular, and intracellular matrices.
- Physical properties of hyaluronic acid are taken advantage of by the tumor cells
    - Compound can create a barrier in the extracellular matrix of the cell, only permitting small molecules like water to pass through.
    - In addition to its largely negative charge, creates a highly hydrodynamic space. Due to the influx of water into the cell, the interstitial volume in tumor cells is increased and this property limits drug penetrance

Aims

Use database query to examine the genetics of cell lines, specifically at alterations in hyaluronic acid synthesis, the hyaluronic acid/CD44 signaling axis, and genes known to be associated with either metastasis or chemotherapy resistance.

- Previous work showed promise for several candidate genes to focus the query on: HAS 1-3, CD44, MGST1, EPHA2, and SGCD.
    - MGST1, EPHA2, and SGCD are candidate early-chemotherapy-response genes that were upregulated in a pilot Sleeping Beauty forward genetic screen that compared the genetics of tumors treated with chemotherapy with untreated tumors
- HAS 1, 2, and 3 had increased expression in chemotherapy-treated samples and have been previously identified as potential contributors to chemotherapy resistance in other cancers.

Methods and Findings

Methods

Using the program R Studio and the package BiocManager, osteosarcoma cell lines were viewed through the Cancer Cell Line Encyclopedia and ones that have alterations in any of the above-mentioned genes were identified. Then further coding was used to search through DepMap identify and eliminate essential genes that are required for life. The result was a list of cell lines in which one or more of our candidate genes would be essential to only those cells. After identifying these cell lines, a program, EnrichR, was used to see if there was validity in the results.

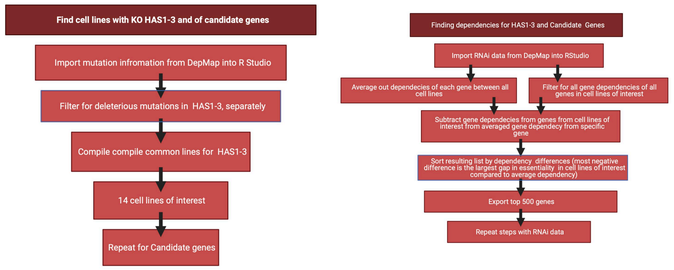

Figure 1: Graphic for overview of project methods

Findings

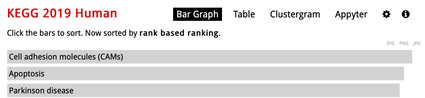

Figure 2: KEGG common characteristics for genes from HAS1-3 KO cell lines

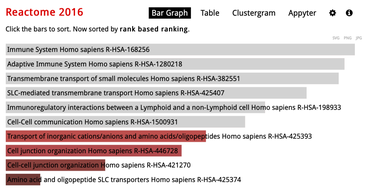

Figure 3: Reactome common characteristics for genes from HAS1-3 KO cell lines

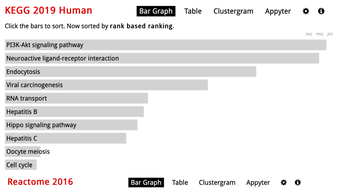

Figure 4: KEGG common characteristics for genes from Candidate genes KO cell lines

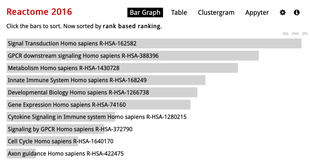

Figure 5: Reactome common characteristics for genes from Candidate genes KO cell lines

Conclusion

Conclusions

There were a total of 10 genes and 11 genes, for HAS1-3 and our candidate genes, respectively, that were found to be essential for those cell lines of interest. After placing these genes into EnrichR, it was found that For the cell lines without HAS1-3, the genes were highly important for immune response and cell adhesion molecules, which directly affect cancer metastasis. For the cell lines without the initial candidate genes, PI3K-AKT signaling as well as general signal transduction were impacted by these genes. This shows that a KO of these genes would result in improper signaling within pathways important for cell growth and signal transduction.

Overall, the results did acknowledge that HAS1-3 and our candidate genes heavily influence osteosarcoma metastasis due to what processes they impact when knocked out.

Future Studies

While this information provides a good starting basis for our research, it does not replace work in the lab. Part of the future directions would include replicating this experiment in the lab setting and comparing the results to this bioinformatics study.
Another future study would be to take this concept, if it proves to be effective, implement it into mouse models to further determine if this is an effective method against Osteosarcoma macroscopic metastases.

Acknowledgements and References

Acknowledgements

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