

IDENTIFYING THE EWING SARCOMA CELL-OF-ORIGIN BY CROSS-SPECIES ENHANCER ACTIVITY ANALYSIS

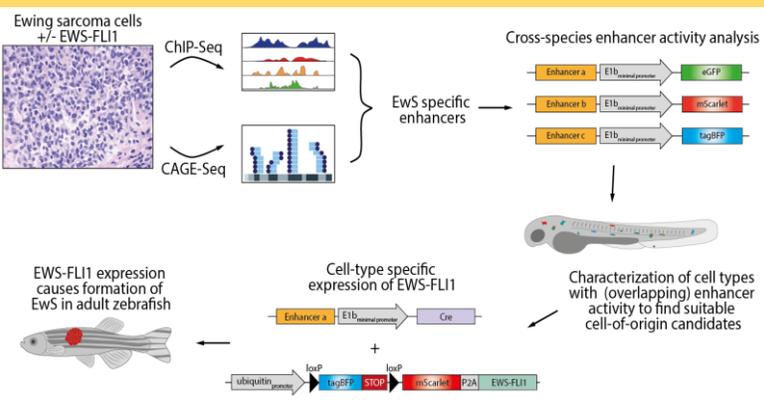


Sarah Grissenberger¹, Andrea Wenninger-Weinzierl¹, Luisa Morelli¹, Magdalena Siller¹, Karin Mühlbacher¹, Heinrich Kovar¹, Christoph Hafemeister¹, Florian Halbritter¹, Martin Distel¹
¹St. Anna Children's Cancer Research Institute, Vienna (Austria)

BACKGROUND

Ewing sarcoma (EwS) is a malignant bone and soft tissue tumor in children and adolescents and 5-year survival for patients with metastatic disease is still <30%. EwS is mainly caused by a chromosomal translocation, leading to the expression of the fusion-oncogene EWS-FLI1. This fusion protein is the sole driver of the disease and leads to massive transcriptional & epigenetic deregulation. An animal model faithfully recapitulating the disease would help to better understand EwS. However, many attempts to genetically model EwS in mouse have failed so far, which is mainly due to the still elusive cell-of-origin. In this project we aim to identify the cellular origin of EwS using an unbiased enhancer-based approach in a cross-species analysis using zebrafish

STRATEGY



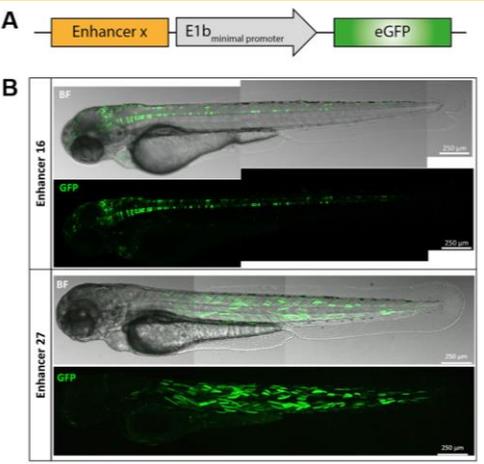
In this project we aim to identify the cellular origin of EwS. Therefore we look at enhancers that are specifically active in this tumor entity by ChIP-Sequencing (H3K27ac & H3K4me1) and by CAGE-Sequencing of tumor cells before and after knockdown of EWS-FLI1. Enhancers that are active after EWS-FLI1 knockdown should also be active in the cell-of-origin. We will visualize cell types with enhancer activity in a cross-species approach using the zebrafish model system. Cell types with enhancer activity will then be characterized in greater detail using scRNA-Sequencing. Once we have identified potential cell-of-origin candidates for EwS we will use a Cre/loxP system to target EWS-FLI1 expression to the respective cell types and monitor all oncogene-expressing zebrafish closely for tumor formation.

CONCLUSION

In summary we have established a setup where we can visualize (human) enhancer activity in zebrafish larvae. We already screened 16 enhancers that are specifically active in EwS cells after knockdown of the fusion oncogene EWS-FLI1. This screen showed two cell types with highly overlapping enhancer activity, being skeletal muscle cells and cells of the ventral central nervous system. Furthermore, we have generated a Cre-inducible effector zebrafish line that expresses EWS-FLI1 and mScarlet upon Cre activation. We showed that oncogene expression is lethal to zebrafish embryos at early developmental stages. To target EWS-FLI1 to a more specific background we generated a construct where Cre can be driven directly by an enhancer from our cross-species analysis and indeed expression of EWS-FLI1 in skeletal muscle cells by enhancer 27 can be tolerated by zebrafish larvae.

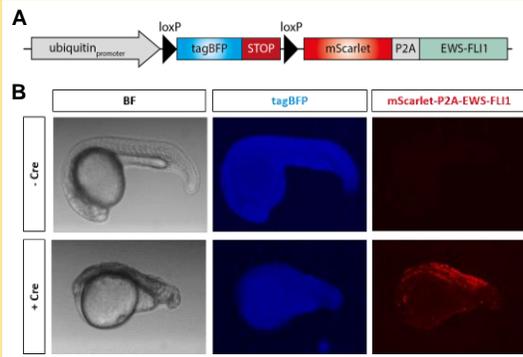
RESULTS

Cross-species enhancer activity analysis



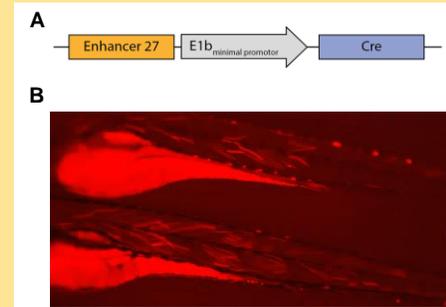
nervous system and enhancer 27 drives reporter expression in skeletal muscle cells (B). So far, we have tested 16 enhancers in our cross-species analysis and all of them showed activity in our assay, suggesting strong conservation of TF binding sites across species. We see that two cell types show a high overlap in enhancer activity, being cells of the ventral central nervous system (floor plate) and skeletal muscle cells. Those cells will now be characterized more closely to see if they could be potential cell-of-origin candidates and tested for their potential to give rise to EwS upon EWS-FLI1 expression.

EWS-FLI1 expression in zebrafish embryos



We established a transgenic zebrafish line that is harboring a Cre-inducible EWS-FLI1 expression construct (A). Upon injection with Cre mRNA mScarlet-P2A-EWS-FLI1 gets expressed in the embryos and this leads to very strong developmental malformations that are not compatible with life, suggesting a strong need to target EWS-FLI1 to the right cellular background.

Targeting EWS-FLI1 to enhancer cell types



In order to target EWS-FLI1 to distinct cell types from our enhancer analysis we generated a construct where Cre is driven by an enhancer in combination with a minimal promoter (A). Using enhancer 27 we can induce EWS-FLI1 expression in skeletal muscle cells (B). This is not lethal to the embryos and all EWS-FLI1-expressing fish will be closely monitored for EwS formation.

Funding:

