High-throughput chemical screen for inhibitors of EWS-FLI1 using a transgenic zebrafish model of Ewing Sarcoma

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Abstract

Ewing Sarcoma is the second most common primary bone tumor of children and adolescents. With a lack of targeted therapies, survival remains poor despite remarkably intense chemotherapeutic regimens. 85% of Ewing cases are caused by a translocation between the EWS gene on chromosome 22 and the FLI1 gene on chromosome 11 [t(11;22)(q24;q12)]. EWS-FLI1 activity can be readily observed in a homozygous transgenic zebrafish model, in which the fusion gene is inserted downstream of the Microphthalmia-Associated Transcription Factor promoter. This localizes EWS-FLI1 expression to melanocytes derived from the neural crest. These fish present with an increased melanocyte count on the dorsum of the head compared to wild type. We hypothesized that compounds which suppress EWS-FLI1 activity will prevent the formation of this phenotype in homozygous zebrafish larvae. A high-throughput chemical screen was performed using two libraries of FDA approved compounds. One compound was found to significantly reduce melanocyte count in EWS-FLI1 homozygous zebrafish compared to unexposed homozygous controls ($p < 0.0001$) in a dose-dependent manner.

Ewing Sarcoma

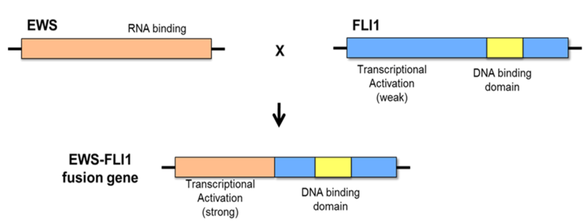
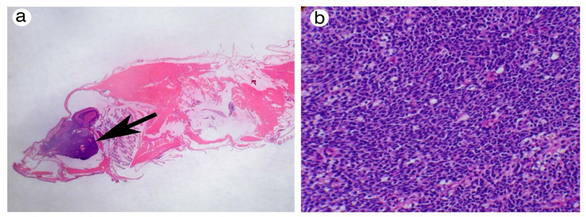


Figure 1: Ewing Sarcoma is a small round blue cell tumor of neurectodermal origin arising most commonly in the pelvis, femur, and humerus. The EWS-FLI1 fusion gene arising from t(11;22) is present in approximately 85% of Ewing Sarcoma cases and encodes a chimeric transcription factor which is a promising candidate for targeted therapies.

Zebrafish Model of Ewing Sarcoma

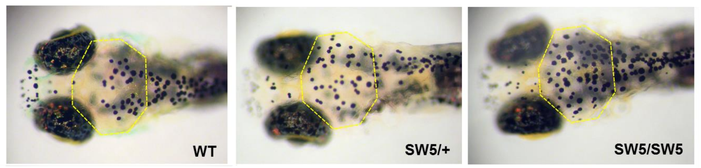


Tg(hsp70l:hEWS-FLI1);p53-/-

Figure 2: The Tol2 transposon system is used to create somatic insertions of human EWS-FLI1, driven by the β-actin or heat shock protein promoter, into both wild-type and p53 null zebrafish (C). Tumors were observed in both genotypes, although there was a much greater tumor incidence in the p53 null line (A). The tumors observed had small, round blue cell histological features similar to human Ewing Sarcoma (B). The gene expression pattern of the zebrafish tumors was also similar to human Ewing Sarcomas (Leacock et al., 2012).

Transgenic expression of EWS-FLI1 from the *mitfa* promotor affects melanocyte development

It is hypothesized that Ewing Sarcoma tumor cells arise from neural crest stem cells. We used the *mitfa* neural crest promotor to drive expression of EWS-FLI1 in our transgenic zebrafish model. tg(*mitfa:EWS-FLI1*)$^{SW5/SW5}$ fish exhibit increased early larval melanocytes, diminished adult pigmentation, and susceptibility to sarcoma development. Inhibition of EWS-FLI1 mRNA translation via antisense morpholino oligonucleotides significantly lowers the early larval melanocyte count, indicating that EWS-FLI1 expression is responsible for the transgenic homozygous phenotype (SW5/SW5).



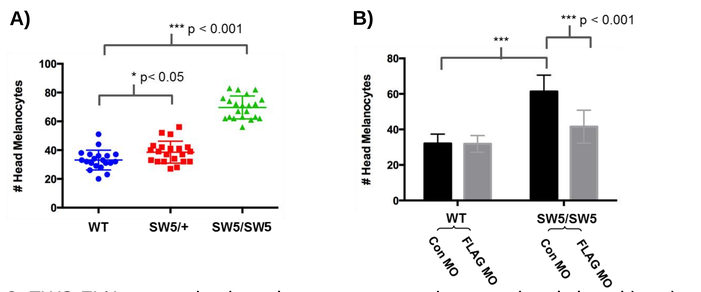
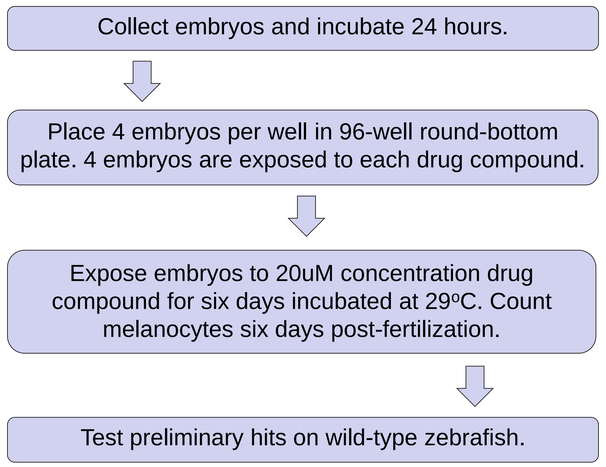


Figure 3: EWS-FLI1 expression in melanocytes causes increased early larval head melanocytes. A) Quantification of melanocytes in region of interest (dotted lines) indicate that 5 dpf homozygous SW5 fish have significantly increased number of melanocytes (avg:69.7±7.9) relative to heterozygous SW5 (avg:38.6±7.7) and wild-type (avg:33.1±6.9) larvae. B) Morpholino knockdown of EWS-FLI1 significantly reduces the number of melanocytes in homozygous SW5 larvae.

Drug Screen Design



Larvae with suppressed EWS-FLI1 activity will exhibit decreased dorsal melanocyte counts at six days post-fertilization.

Compound 3D7 Suppresses EWS-FLI1 expression

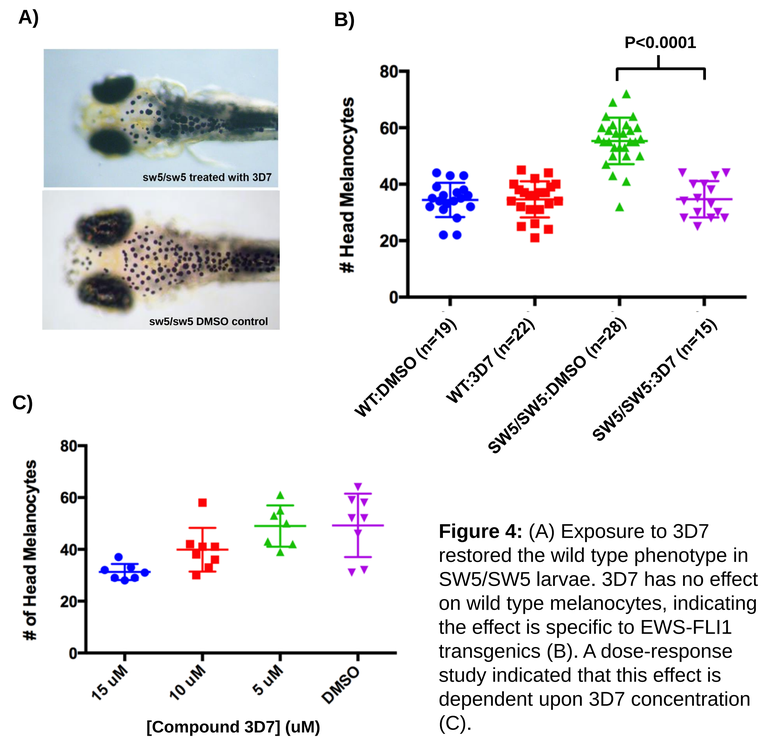


Figure 4: (A) Exposure to 3D7 restored the wild type phenotype in SW5/SW5 larvae. 3D7 has no effect on wild type melanocytes, indicating the effect is specific to EWS-FLI1 transgenics (B). A dose-response study indicated that this effect is dependent upon 3D7 concentration (C).

Discussion

From these results, we conclude that 3D7 interferes with EWS-FLI1 activity in the tg(*mitfa:EWS-FLI1*) homozygous zebrafish model. Further testing of this compound will be performed in Ewing sarcoma cell lines to observe its potential anti-tumorigenic or apoptotic capabilities. Investigation into the mechanisms of EWS-FLI1 activity suppression via 3D7 exposure may uncover novel strategies for targeted therapy of Ewing Sarcoma.

Acknowledgments

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References

1. Leacock, et al.. A zebrafish transgenic model of Ewing's sarcoma reveals conserved mediators of EWS-FLI1 tumorigenesis. DMM, 2012.