Growth factor production for enhanced growth of fish multipotent stem cells for cell-cultivated meat

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Abstract

The growth factor fibroblast growth factor 2 (FGF2) regulates a variety of cellular functions, including cell proliferation, differentiation, and migration. As such, FGF2 is commonly used to maintain and promote the growth of induced pluripotent stem cells (iPSCs) in culture. FGF2-G3, a recently developed mutation of the native FGF2 sequence[1], further improves FGF2's stability and potency at typical cell culture temperatures, reducing protein usage and need for medium changes in cell passaging schedules. But while human FGF2 has been extensively studied in the context of mammalian cell culture, its performance in the culture of fish-derived cells has not, and nor has the comparative performance of FGF2 native sequences derived from fish species. Such data would be valuable in enabling fish stem cell culture for basic research, with clear applications in veterinary medicine and cell-cultivated meat. We hypothesized that FGF2 proteins derived from various species of fish will better facilitate the growth of fish stem cells while decreasing off-target differentiation, compared with native human FGF2. FGF2 protein was expressed recombinantly in *E. coli*, purified, and evaluated for thermostability (via a SYPRO Orange thermal shift assay) and function (via 3T3 cell proliferation). The resulting data highlight the effect of fish sequence change on protein stability and performance in mammalian cell culture, with further studies pending that utilize proliferating fish cells to evaluate changes in functional effect.

Acknowledgements

California State University SAN MARCOS

gfi Good Food Institute

References

[1]Dvorak P, Bednar D, Vanacek P, Balek L, Eiselleova L, Stepankova V, Sebestova E, Kunova Bosakova M, Konecna Z, Mazurenko S, Kunka A, Vanova T, Zoufalova K, Chaloupkova R, Brezovsky J, Krejci P, Prokop Z, Dvorak P, Damborsky J. Computer-assisted engineering of hyperstable fibroblast growth factor 2. Biotechnol Bioeng. 2018 Apr;115(4):850-862. doi: 10.1002/bit.26531. Epub 2018 Jan 24. PMID: 29278409.

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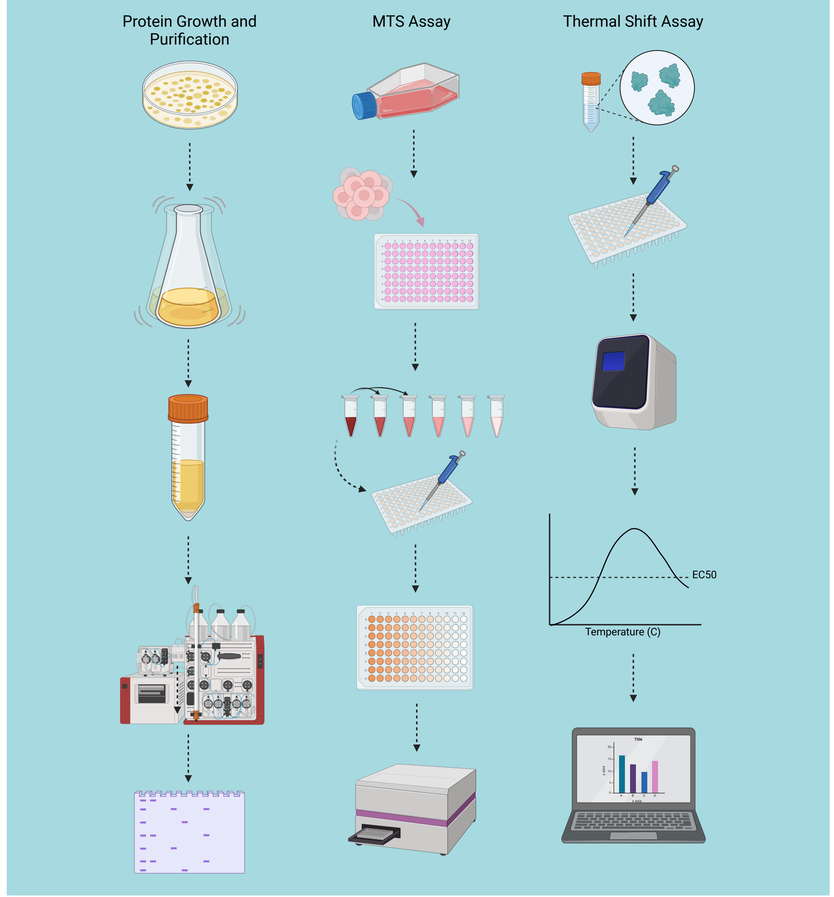
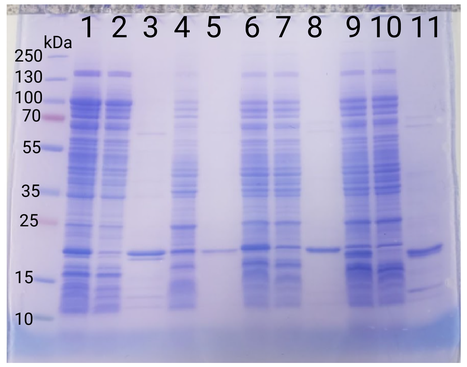
Workflow for FGF2 Homolog Testing

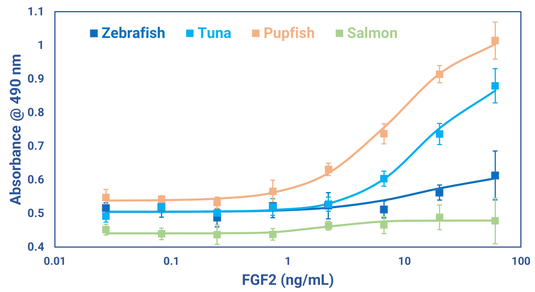


Protein Purification



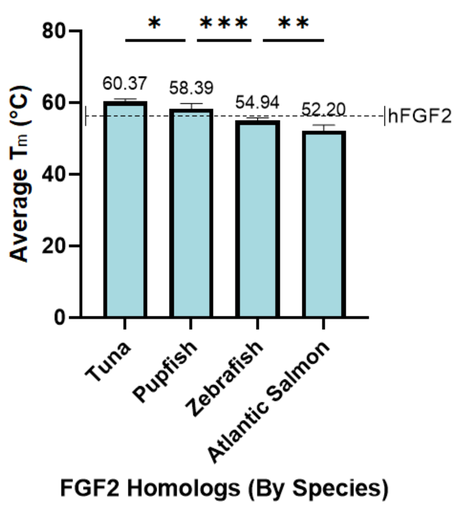
SDS-PAGE analysis of purified FGF2 homologs, including load, flowthrough, and elution samples (respectively) from small scale Ni IMAC purification. Homologs shown include pupfish (1-3), yellowfin tuna (4-5; flowthrough excluded), atlantic salmon (6-8), and zebrafish (9-11). On the left is the molecular weight ladder (masses in kDa).

Fibroblast Outgrowth Assay



An initial screen of FGF2 homologs from zebrafish, yellowfin tuna, sheepshead minnow, and atlantic salmon demonstrated variable performance in initial 3T3 outgrowth assays, evaluated via MTS (n=6). From ISSCR 2023.

Thermal Shift Assay



Average melting temperature (Tm) of each homolog, calculated using a SYPRO Orange Thermal Shift Assay (Bio-Rad) against No-protein (buffer) controls.

- Data reflect n=3 replicates per assay over two independent protein lots (n=6)
- Dotted line indicates average T_M calculated from independent protein lots for human FGF2
- Error bars indicate standard deviation
- Asterisks indicate mean analysis by one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001)
- Comparisons not shown by one-way ANOVA are also significantly different (p<0.001)

Discussion

Conclusions

- Piscine FGF2s have comparable stability with human FGF2
- Some homologs show higher T_m, and some lower, than human native FGF2
- All proteins show lower Tm compared with engineered FGF2-G3 (T_m>78°C)
- 3T3 cells can be used to evaluate piscine FGF2, but may be limited (not a piscine cell line)

Future Directions

- Test proteins in piscine multipotent stem cell cultures
- Repeat conditions for further testing and comparison with mammalian homologs