5-2019

Comparing the Effects of Fibroblast Growth Factors on Growth Rate of Human Fibroblast Cell Lines

Tasha Repella

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An undergraduate honors thesis
submitted to the
Department of Biomedical Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

April 23, 2019

by

Tasha Repella

Honors Advisor: Dr. Raj Rao

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Abstract

Numerous studies have demonstrated the usefulness of growth factors in medium formulations to promote proliferation and viability of human fibroblasts in laboratory settings. However, due to the differences in the source and age of fibroblasts, the ability of growth factors like hFGF1 to promote proliferation varies. Equally difficult is the ability of hFGF1 to promote proliferation of diseased fibroblasts due to complexities associated with specific mitochondrial diseases. In this context, we decided to evaluate the potential for novel hyper stable growth factors super hFGF and hFGF1 (shFGF and hFGF1 respectively), to contribute to proliferation of different normal and diseased fibroblasts. In studies using different concentrations of shFGF and hFGF1, the growth rate of normal and diseased fibroblasts was determined. Our results indicate that shFGF treatment contributed to the highest increase in cell proliferation across all three cell lines, with the greatest effect on normal fibroblasts. A general trend of increase in cell number was observed in the diseased fibroblasts upon shFGF treatment. Future studies will focus on a broader evaluation of a combination of FGF variants in cell culture medium formulations in promoting proliferation of diseased fibroblasts towards contributing to an overall improvement in cellular and biochemical function in the diseased state.

Introduction

Studies have shown that by culturing human adipose derived stem cells (hADSC) with a basic fibroblast growth factor (bFGF) and an epidermal growth factor (EGF) at varying concentrations (0–10 ng/ml), an increased proliferation of hADSCs occurred up to three-fold over a time span of 7–8 days.[1] Similar studies have also shown that human dermal fibroblasts (HDFs) exhibited an increased cell growth rate, improved proliferative capacity and decreased doubling time when cultured in medium containing additional FGFs.[2]

Human acidic fibroblast growth factor 1 (hFGF1) is a protein recognized with regards to improvement of cell growth and tissue repair.[3] It is a type of polypeptide recognized as a strong mitogen, involved in cell proliferation, cell differentiation and wound healing processes.[3, 4] This growth factor is unlike other members of its family as it is an ideal target for therapeutic applications in culture. It is useful regarding this research as it possesses broad capabilities in regenerative medicine applications including tissue repair, cell growth and embryonic development.[5] Variations of the hFGF1 (super, wild-type, and R-136) have been developed with various characteristics slightly affecting the structure, stability and even heparin-binding affinity leading to differences in cell proliferation that we observe when implemented into cell culture.[3] Based on these observations, we have proposed the addition of these novel FGF variants as a means of improving the cell growth rate of difficult-to-propagate diseased SBG3 and SBG8 fibroblast cell lines.

Materials and Methods

Cell lines and cell culture. Primary culture of health control BJ human foreskin fibroblast (ATCC, #CRL-2522, Manassas, VA, USA), SBG3 (point mutation at complex V, ATP6 subunit) and SBG8 (whole region of mitochondrial DNA deleted) were maintained in Fibroblast medium (MEM w/s 10% FBS and 2mM Glutamine). Fibroblasts were briefly expanded via 0.05% trypsin enzymatic passaging. The diseased fibroblasts containing specific defects in the mitochondrial genome were obtained from the lab of Dr. Iyer for use in this study.

Evaluation of Cell Proliferation. Briefly, all the cell lines (BJ, SBG3 and SBG8) were propagated in T75 flasks and cultured till required number of cells for use in experimentation was acquired. Twelve hours prior to initiating the use of cells, the fibroblast medium was replaced with medium without FBS, in order to ensure uptake of FGF variants in the study. Initially, the cells were seeded at a concentration of 20,000 cells/well in a 6-well plate with addition of different concentrations of shFGF or hFGF1 (Figure 1). Each experiment was conducted in triplicate across three passages. The fibroblast medium along with the appropriate FGF was changed every day. At the end of the 7 days, cells from each well was harvested using 0.05% trypsin. An aliquot of cells was stained with trypan blue and total number of cells per well was determined using a hemocytometer. The purpose of the experiments was to assess the effects of shFGF and hFGF1 on the growth rate of the three different fibroblasts.

Fibroblasts	shFGF (ng/ml)	shFGF (ng/ml)	shFGF (ng/ml)	shFGF (ng/ml)	hFGF1 (ng/ml)	hFGF1 (ng/ml)	hFGF1 (ng/ml)	hFGF1 (ng/ml)
BJ	0	100	250	500	0	100	250	500
SBG3	0	100	250	500	0	100	250	500
SBG8	0	100	250	500	0	100	250	500

Figure 1. Schematic of experimental design to assess effects of FGF on cell growth rate

Results and Discussion

Given these diseased states, we have previously observed that both SBG3 and SBG8 fibroblasts continuously demonstrated poor performance in cell culture with growth rate and cells/cm^2 upon confluence. They thus served as ideal cell lines to demonstrate the potential of the novel hyperstable growth factors (super hFGF) to promote cell survival and proliferation.

As shown in Table 1-3, shFGF was determined to significantly promote increased proliferation of BJ fibroblast and diseased SBG3 and SBF8 cells. In general, it was observed that a concentration of 250ng/ml was sufficient to promote proliferation of all the cell lines analyzed.

Table 1: Summary of results from growth rates for BJ fibroblast Cell line.

	Cell Line (ng/mL)	Day 0 Count	Day 7 Count
Trial 1	**BJ hFGF1control**	20,000	262,000
	BJ shFGF control	20,000	270,000
	BJ hFGF1100	20,000	330,000
	BJ hFGF1 250	20,000	495,000
	BJ hFGF1 500	20,000	517,000
	BJ shFGF 100	20,000	652,000
	BJ shFGF 250	20,000	720,000**
	BJ shFGF 500	20,000	525,000
Trial 2	**BJ hFGF1 control**	20,000	300,000
	BJ shFGF control	20,000	292,000
	BJ hFGF1100	20,000	450,000
	BJ hFGF1 250	20,000	495,000
	BJ hFGF1 500	20,000	577,500
	BJ shFGF 100	20,000	742,000
	BJ shFGF 250	20,000	990,000
	BJ shFGF 500	20,000	1,072,500**
Trial 3	**BJ hFGF1 control**	20,000	127,500
	BJ shFGF control	20,000	150,000
	BJ hFGF1 100	20,000	247,500
	BJ hFGF1250	20,000	480,000
	BJ hFGF1500	20,000	502,500
	BJ shFGF 100	20,000	945,000
	BJ shFGF 250	20,000	1,112,500
	BJ shFGF 500	20,000	1,162,500**

****Cultures exhibiting largest growth**

Table 2: Summary of results from growth rates for SBG3 Fibroblast Cell line

	Cell Line (ng/mL)	Day 0 Count	Day 7 Count
Trial 1	**SBG3 hFGF1 control**	20,000	82,500
	SBG3 shFGF control	20,000	90,000
	SBG3 hFGF1 100	20,000	112,500
	SBG3 hFGF1 250	20,000	165,000**
	SBG3 hFGF1 500	20,000	150,000
	SBG3 shFGF 100	20,000	120,000
	SBG3 shFGF 250	20,000	135,000
	SBG3 shFGF 500	20,000	150,000
Trial 2	**SBG3 hFGF1 control**	20,000	120,000
	SBG3 shFGF control	20,000	97,500
	SBG3 hFGF1 100	20,000	165,000
	SBG3 hFGF1250	20,000	202,500
	SBG3 hFGF1500	20,000	180,000
	SBG3 shFGF 100	20,000	195,000
	SBG3 shFGF 250	20,000	232,000**
	SBG3 shFGF 500	20,000	172,500
Trial 3	**SBG3 hFGF1control**	20,000	105,000
	SBG3 shFGF control	20,000	120,000
	SBG3 hFGF1100	20,000	270,000
	SBG3 hFGF1250	20,000	352,500
	SBG3 hFGF1500	20,000	360,000
	SBG3 shFGF 100	20,000	397,500
	SBG3 shFGF 250	20,000	405,000**
	SBG3 shFGF 500	20,000	120,000

****Cultures exhibiting largest growth**

Table 3: Summary of results from growth rates for SBG8 Fibroblast Cell line

	Cell Line (ng/mL)	Day 0 Count	Day 7 Count
Trial 1	**SBG8 hFGF1 control**	20,000	75,000
	SBG8 shFGF control	20,000	60,000
	SBG8 hFGF1 100	20,000	90,000
	SBG8 hFGF1 250	20,000	82,500
	SBG8 hFGF1 500	20,000	82,500
	SBG8 shFGF 100	20,000	112,500
	SBG8 shFGF 250	20,000	187,000**
	SBG8 shFGF 500	20,000	90,000
Trial 2	**SBG8 hFGF1 control**	20,000	112,500
	SBG8 shFGF control	20,000	97,500
	SBG8 hFGF1 100	20,000	97,500
	SBG8 hFGF1 250	20,000	112,500
	SBG8 hFGF1 500	20,000	150,000**
	SBG8 shFGF 100	20,000	82,500
	SBG8 shFGF 250	20,000	135,000**
	SBG8 shFGF 500	20,000	135,000
Trial 3	**SBG8 hFGF1 control**	20,000	52,500
	SBG8 shFGF control	20,000	52,500
	SBG8 hFGF1 100	20,000	90,000
	SBG8 hFGF1 250	20,000	97,500
	SBG8 hFGF1 500	20,000	112,500
	SBG8 shFGF 100	20,000	135,000**
	SBG8 shFGF 250	20,000	120,000
	SBG8 shFGF 500	20,000	120,000

****Cultures exhibiting largest growth**

As shown in Figure 2 below, treatment with shFGF1 had a significant effect on the proliferation of BJ fibroblast cell line with a 4-fold increase in cell numbers. Although the effect of shFGF on the two diseased lines (SBG3, SBG8) was not as pronounced, we did observe an increase in cell number, a marked difference when compared to how the cell lines behaved in our previous studies.

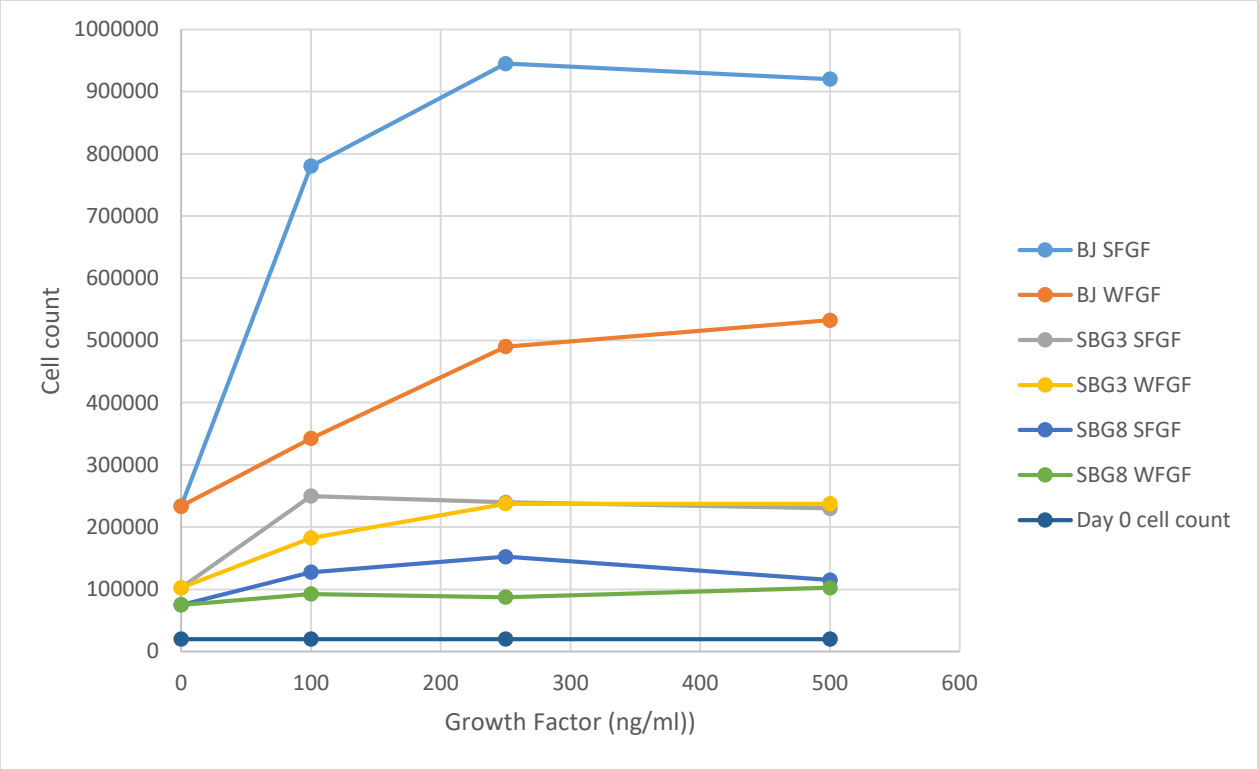

Figure 2. Cell Proliferation of Fibroblast Cell Lines with Respective Growth Factors

We conducted statistical analysis to demonstrate the effects of shFGF1 and wFGF on all cell lines. Cells treated with shFGF displayed higher proliferation compared to those treated with hFGF1. However, shFGF treatment was not consistent enough to determine which was best. Although it appears shFGF may be the best additive, statistical analysis demonstrated that using a concentration of 250 ng/mL is best concentration FGF as 500 ng/mL seems to be an overdose. Figures 3-5 depicting statistical analysis across the different shFGF and hFGF1 treatments for the 3 cell lines.

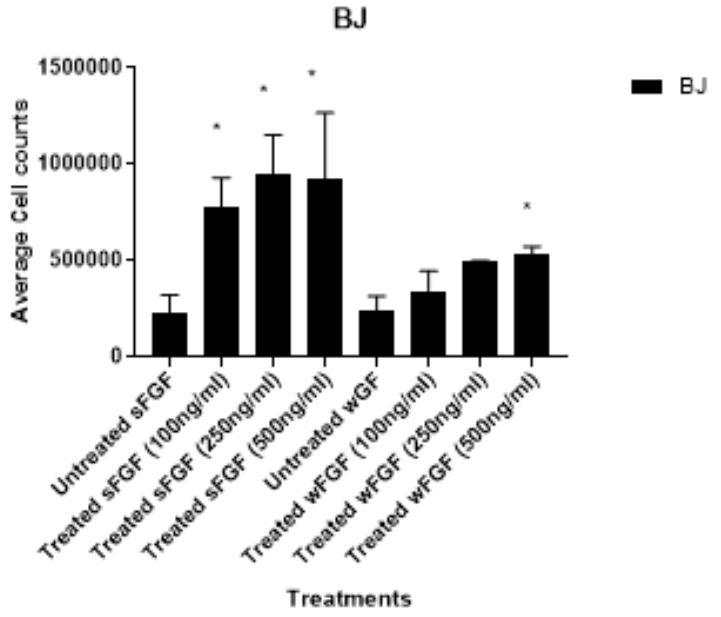

Figure 3. Statistical analysis of increased proliferation of FGFs on BJ cell line.

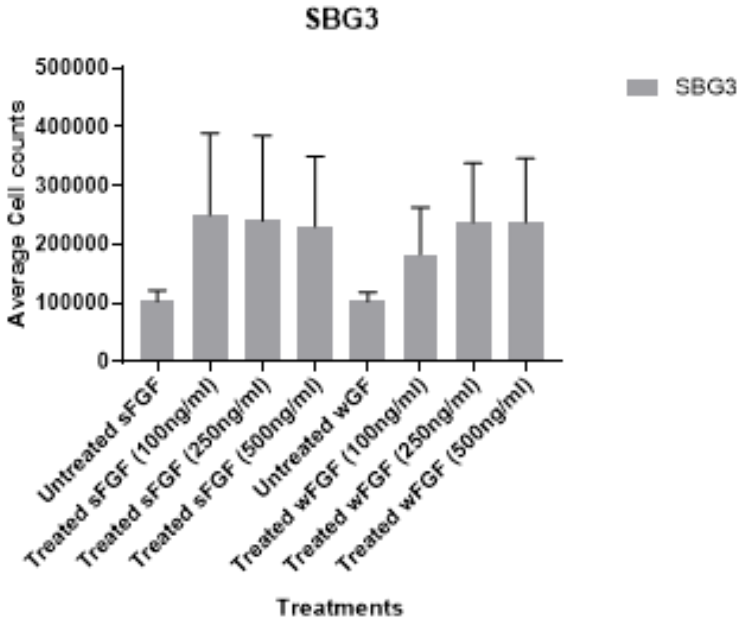

Figure 4. Statistical analysis of increased proliferation of FGFs on SBG3 cell line.

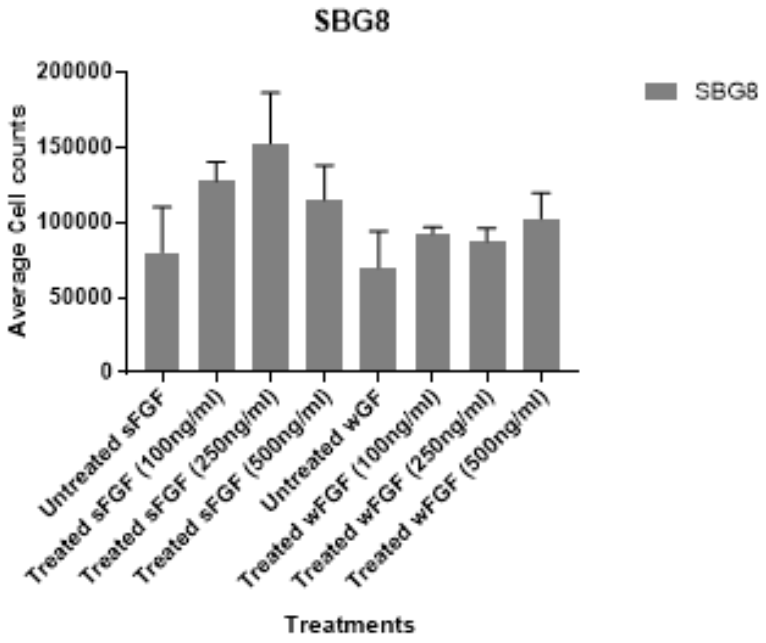

Figure 5. Statistical analysis of increased proliferation of FGFs on SBG8 cell line.

Our results indicate that proliferation increase may only be significant for BJ fibroblast upon shFGF1 treatment. The hFGF1 does not have a significant effect on either experimental line, but shFGF is significant with increased proliferation of all three cell lines.

Conclusion

Based on our results, we can conclude that introduction of FGFs increased cell proliferation by 2-4 fold. Treatment with shFGF1 contributed to higher increase in cell proliferation than those treated with hFGF1. SBG3 experienced higher proliferation than SBG8 with FGF influence. Statistical analysis indicates that treatment with shFGF1 significantly contributed to increased proliferation in only the BJ fibroblast, while it contributed a moderate increase in proliferation for the two diseased fibroblasts. Overall, novel fibroblast growth factors

like shFGF1 may be used in the proliferation of fibroblasts and used in the development of defined proliferation medium.

For future studies, variations from experimental cell lines need to be eliminated with further testing and improved parameters. We also propose experiments to test the effect of shFGF on stem cells and other cell lines that do not contain genetic abnormalities. This research will continue by observing proliferation effects on the experimental cell lines using shFGF1 and hFGF1 with other FGF-variants

Acknowledgements

First, I would like to thank Josh Stabach for his many hours of mentorship in the lab throughout this research. This work would also not have been possible without Dr. Suresh Thallapuranam and his development of the novel growth factors used on the cell lines. Thank you to Dr. Shilpa Iyer for providing oversight while working in the lab, and a special thanks to my advisor Dr. Raj Rao along with Dr. Srikanth Sivaraman both of whom offered valuable guidance and insight along the way.

References

1. Hebert, Teddi L., Xiying Wu, Gang Yu, Brian C. Goh, Yuan-Di C. Halvorsen, Zhong Wang, Cedric Moro, and Jeffrey M. Gimble. "Culture Effects of Epidermal Growth Factor (EGF) and Basic Fibroblast Growth Factor (bFGF) on Cryopreserved Human Adipose-derived Stromal/stem Cell Proliferation and Adipogenesis." *Journal of Tissue Engineering and Regenerative Medicine*3, no. 7 (2009): 553-61. doi:10.1002/term.198.
2. Abdian, Narges, Payam Ghasemi-Dehkordi, Morteza Hashemzadeh-Chaleshtori, Mahbobe Ganji-Arjenaki, Abbas Doosti, and Beheshteh Amiri. "Comparison of Human Dermal Fibroblasts (HDFs) Growth Rate in Culture Media Supplemented with or without Basic Fibroblast Growth Factor (bFGF)." *Cell and Tissue Banking*16, no. 4 (2015): 487-95. Doi:10.1007/s10561-015-9494-9.
3. Davis, Julie Eberle, Arwa Alghanmi, Ravi Kumar Gundampati, Srinivas Jayanthi, Ellen Fields, Monica Armstrong, Vanessa Weidling, Varun Shah, Shilpi Agrawal, Bhanu Prasanth Koppolu, David A. Zaharoff, and Thallapuranam Krishnaswamy Suresh Kumar. "Probing the Role of Proline −135 on the Structure, Stability, and Cell Proliferation Activity of Human Acidic Fibroblast Growth Factor." *Archives of Biochemistry and Biophysics*654 (July 19, 2018): 115-25. doi:10.1016/j.abb.2018.07.017.
4. Ornitz, David M., and Nobuyuki Itoh. "The Fibroblast Growth Factor Signaling Pathway." *Wiley Interdisciplinary Reviews: Developmental Biology*4, no. 3 (2015): 215-66. doi:10.1002/wdev.176.
5. "Fibroblast Growth Factor 1 (FGF1)." Science-Business EXchange 7, no. 31 (April 15, 2019): 922. doi:10.1038/scibx.2014.922.