Novel Engineered Basic Fibroblast Growth Factor Improves Stability and Enables Improved Cell Culture Outcomes

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Introduction

HS bFGF: Engineered for greater stability

- Basic fibroblast growth factor (bFGF) is used in NSC media to maintain multipotency and is known to be present in the tumor microenvironment
- **Native bFGF rapidly loses biological activity** when exposed to culture conditions (37°C); we found only ~20% bioactivity after 72 hours
- **HS bFGF maintains > 90% homology** to the native protein **and ≥ 80% biological activity**, even after 72 hours of exposure to 37°C

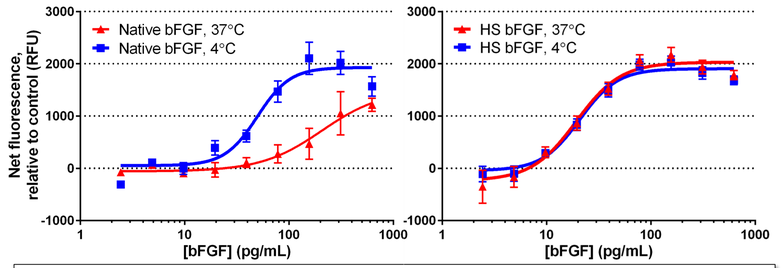


Figure 1. HS bFGF maintained 95% activity after 72 hours at 37°C. Dose-response of Balb/3T3 mouse embryonic fibroblast cells to native (top) and HS (bottom) bFGF stored at 4°C or 37°C for 72 hours. Analysis by PrestoBlue® assay after 18 h stimulation. Mean ± SEM.

Human Embryonic Stem Cell-Derived NSC Culture

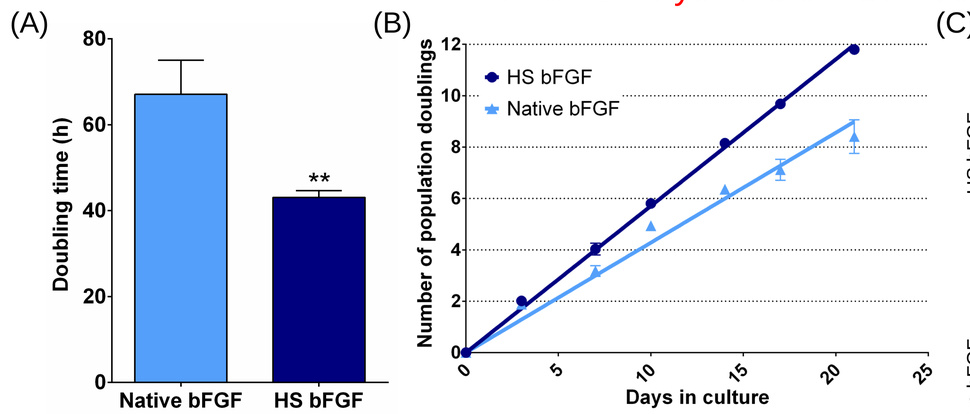


Figure 4. HS bFGF allows for normal proliferation of multipotent NSCs with less bFGF. At 5 ng/mL, HS bFGF (A) reduced the doubling time by ~30% (back to 20 ng/mL standard, ~45 h) and (B) had more cells at each passage versus native bFGF at the same concentration, (C) while maintaining a multipotent morphology and SOX1 expression. Mean ± SEM. ** = $p < 0.01$. Arrows denote neurite outgrowth.

Primary Rat Neural Stem Cell (NSC) Culture

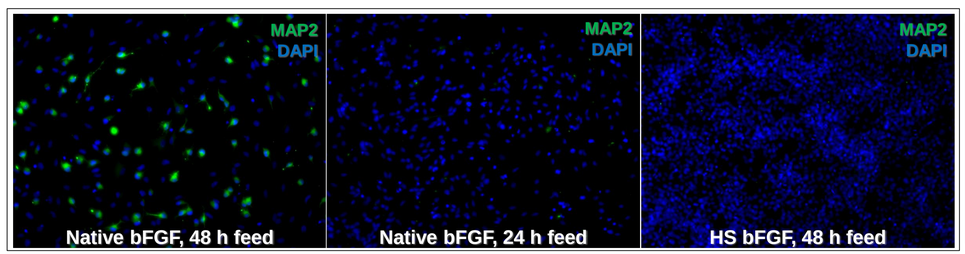
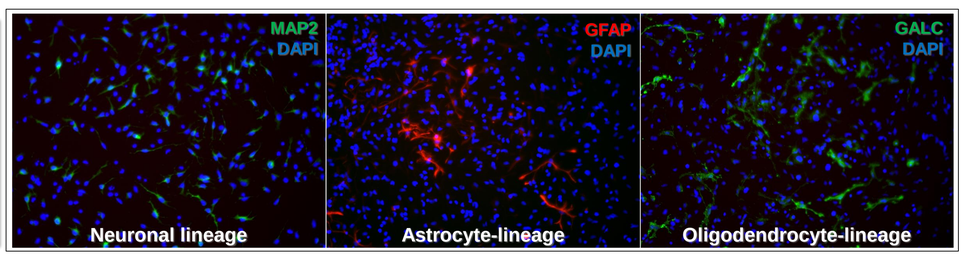


Figure 2. HS bFGF maintains multipotent NSCs with fewer feeds. Using 10 ng/mL bFGF, HS bFGF decreased the doubling time and maintained NSC multipotency with feeds every 48 h.

Figure 3. HS bFGF does not impact NSC differentiation. Three days after the removal of HS bFGF, the NSCs were stained for differentiation markers and showed trilineage potential.



Human Breast Cancer Spheroid Culture

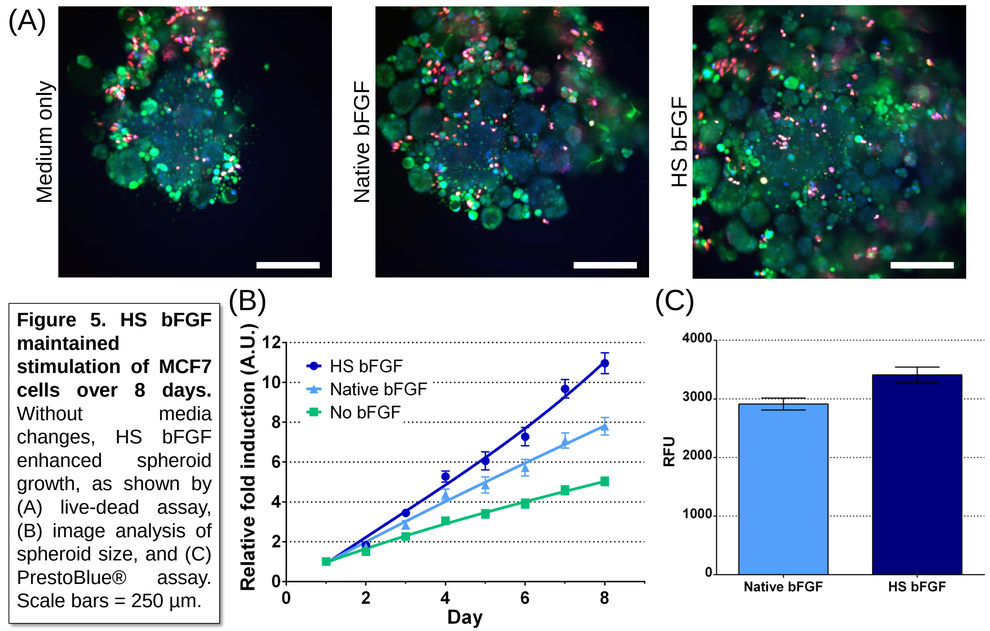


Figure 5. HS bFGF maintained stimulation of MCF7 cells over 8 days. Without media changes, HS bFGF enhanced spheroid growth, as shown by (A) live-dead assay, (B) image analysis of spheroid size, and (C) PrestoBlue® assay. Scale bars = 250 µm.

Conclusions

- In primary rat NSCs, using **HS bFGF allows for a more user-friendly workflow** while maintaining multipotency
- In human ESC-derived NSCs, HS bFGF can maintain multipotency and standard doubling times with **reduced bFGF concentrations**
- After expansion, **HS bFGF can be removed just as easily as native bFGF** to allow for downstream differentiation into neurons and glial cells
- **HS bFGF can be used for spheroid culture,** or other systems where media changes are undesirable

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