Induction of cancer cell migration using a heat stable bFGF

Introduction

Much of today's cancer research focuses on stopping the spread of cancer cells from the primary tumor site to secondary sites, a process known as metastasis. In a laboratory setting, this is often modeled by the induction of cancer cell migration in a wound healing assay (also referred to as a "scratch assay"). The ability of cancer cells to respond to a chemical signal (i.e., chemokine) and heal a damaged cell monolayer *in vitro* is indicative of their invasiveness or metastatic potential. This assay may also be used to test the ability of novel anticancer therapeutics to block chemokine-induced migration. When assessing the migratory potential of cells, it is important to have a growth factor that does not rapidly degrade in standard culture conditions. Basic fibroblast growth factor (bFGF) is a physiologically relevant growth factor, but the native protein is not stable at physiological temperatures (i.e., 37˚C). Gibco™ Heat Stable Recombinant Human bFGF Protein (HS bFGF, Cat. No. PHG0367) offers a solution to this problem. HS bFGF elicits the same cellular response as native bFGF. However, whereas native bFGF degrades over the course of an experiment, adding undesired variability to the system, HS bFGF is engineered to maintain bioactivity at 37˚C (Figure 1). In this application note, we demonstrate the utility of HS bFGF in a typical wound healing assay with the breast adenocarcinoma MCF7 cell line.

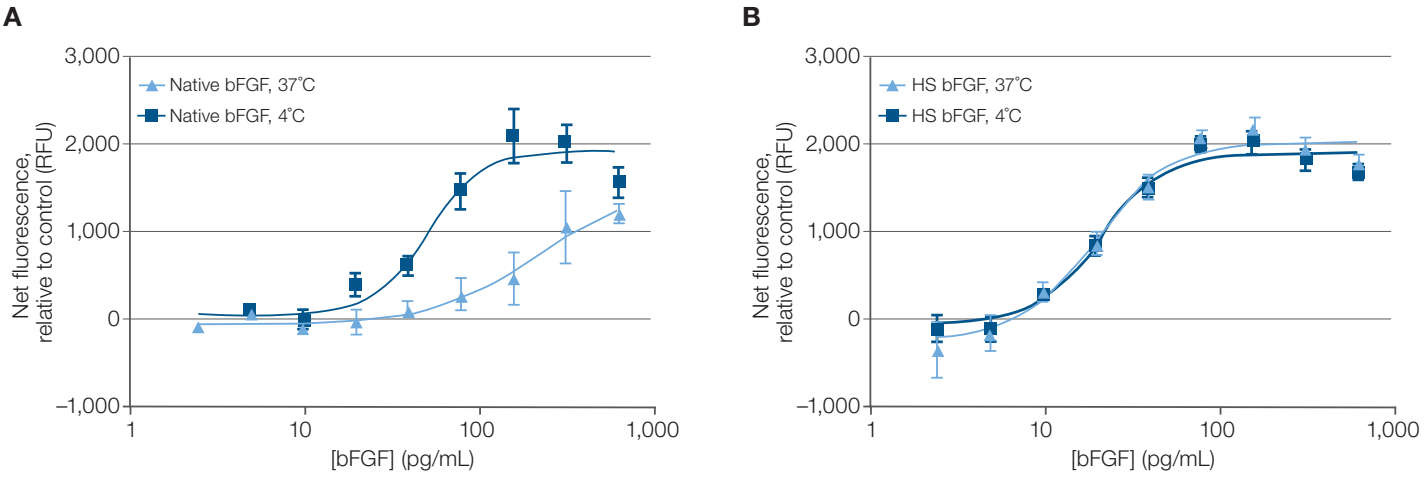

Figure 1. HS bFGF is engineered to sustain bioactivity at standard culture conditions. Activity of native bFGF **(A)** and HS bFGF **(B)** after 72 hours at 37°C (light blue), as compared against the control (dark blue) at 4˚C. Activity measured by mouse BALB/3T3 proliferation using Invitrogen™ PrestoBlue™ Cell Viability Reagent, reported as the mean ± standard error of the mean (SEM).

Materials and methods

The recommended workflow is typical of a wound healing assay; no special modifications are required to use HS bFGF. The generalized workflow utilized for the MCF7 wound healing assay is outlined in Figure 2. The MCF7 cells were seeded at a high density to facilitate full coverage of the growth area (100% confluency). The cells were allowed to attach for three hours in growth medium containing 10% fetal bovine serum (FBS) at 37˚C, 5% CO_2 (standard culture conditions). After three hours, the medium was changed to a low-serum (LS) medium (i.e., 1% FBS) to serum-starve the cells overnight. The next morning, the cells were imaged to document the monolayer pre-scratch (100% confluency). Afterwards, a small pipette tip was used to scratch through the center of each well. The wells were then washed with LS medium once to clear away the dislodged cells and the experimental conditions were then applied. For this study, the experimental conditions were:

- LS medium (negative control)
- LS medium containing 10 ng/mL of HS bFGF (Cat. No. PHG0367)

Under the experimental conditions, the cells were incubated at standard culture conditions and imaged every 3 hours. The confluency over time was analyzed to assess the dynamics of wound closure under the varying treatment conditions.

Results

We found that HS bFGF significantly increased the rate of wound closure (i.e., recovery from the wound) as compared to the negative control (Figure 3). The ability of the HS bFGF to enhance wound closure is evident from both the photomicrographs and the image analysis that were used to evaluate wound closure over time (Figure 3). Over the course of the 72-hour experiment, not only did the MCF7 cells exposed to HS bFGF close the wound more quickly, but also more completely than the cells exposed only to LS medium. This indicates that heat stable bFGF is enhancing migration in MCF7 cells.

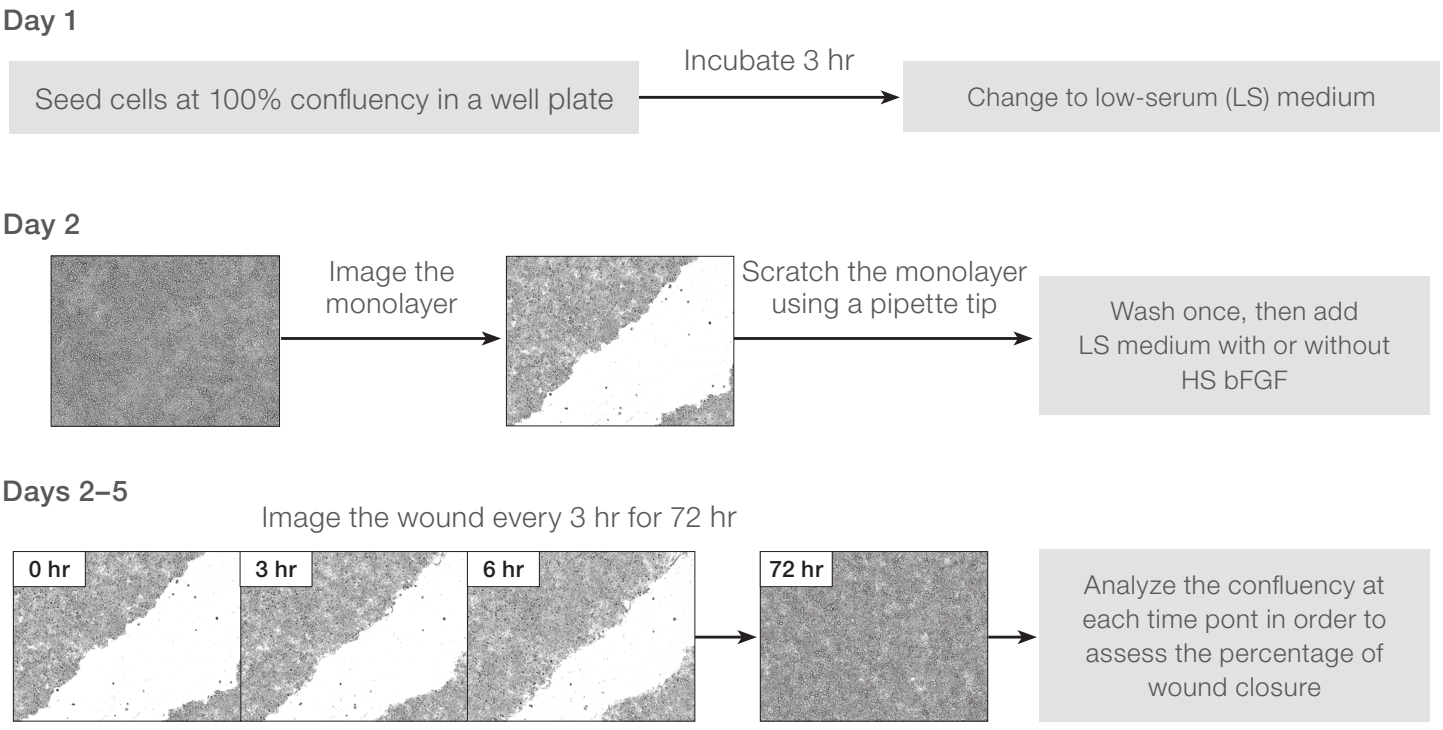

Figure 2. Suggested workflow for wound healing assay. MCF7 cells were seeded at full confluency and serum-starved (day 1). Confluent monolayers were then imaged before scratching, scratched to create a cell-free area, washed, and treated with LS medium with or without 10 ng/mL HS bFGF under standard culture conditions (day 2). The effect of the treatments on the rate of wound closure was monitored every 3 hours for 72 hours (days 2–5).

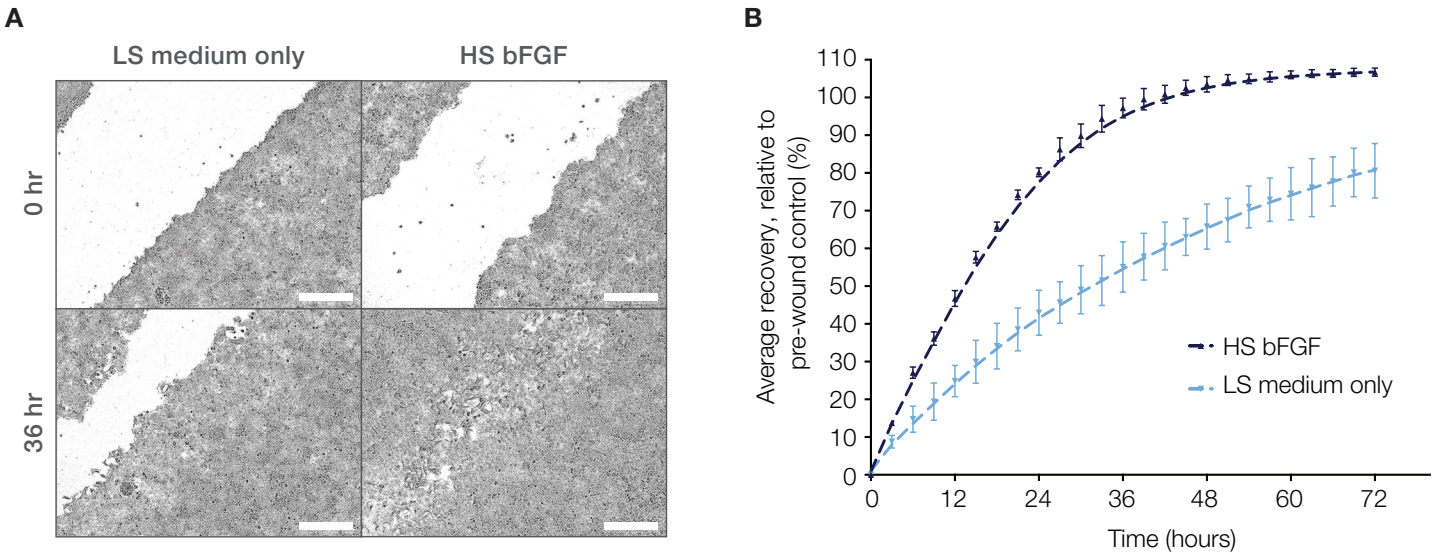

Figure 3. HS bFGF enhances wound healing relative to control. (A) Background-subtracted photomicrographs of MCF7 wound healing; scale bars = 300 µm. **(B)** Normalized recovery from wound over time (relative to the pre-wound control), based on image analysis of confluence, reported as the mean ± SD.

Conclusions

Heat Stable Recombinant Human bFGF Protein is a chemokine, or causes a migratory response, for MCF7 cells. In addition, the heat stability of HS bFGF deems it appropriate for experiments involving long-term culture where bioactivity degradation is undesirable. With these attributes, HS bFGF may be useful in broad areas of cancer research, such as in the evaluation of novel anti-metastasis compounds for a range of cancers.

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Ordering information

Product	Cat. No.
Heat Stable Recombinant Human bFGF Protein, 5 µg	PHG0367
Heat Stable Recombinant Human bFGF Protein, 50 µg	PHG0368
Heat Stable Recombinant Human bFGF Protein, 100 µg	PHG0369
Heat Stable Recombinant Human bFGF Protein , 500 µg	PHG0360
DMEM, high glucose, GlutaMAX Supplement, pyruvate	10569
Fetal Bovine Serum, certified, US origin	16000
DPBS, no calcium, no magnesium	14190
TrypLE Express (1X)	12604
Nunc EasYFlask Cell Culture Flasks	156499
Nunc Cell-Culture Treated Multidishes	152640
EVOS FL Auto 2 Imaging System	AMAFD2000
EVOS Onstage Incubator	AMC1000

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