Research Networking Programmes

Short Visit Grant ☐ or Exchange Visit Grant ☒

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Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Real-time control of ERK activation dynamics to fine tune cell fate

Application Reference N°: 4974

1) Purpose of the visit

The aim of this research proposal is incremental, and proposes to explore how real-time control of extracellular response kinase (ERK) activation dynamics might enable to buffer against cellular noise induced by the environment to fine tune cell fate determination in a cell population.

2) Description of the work carried out during the visit.

FGFs (fibroblast growth factors) are known as the key manipulator for the cell fate decision. Especially, they are essential for maintaining self-renewal in human embryonic stem cells and induced pluripotent stem cells. (Xu C et al., 2005, Stem Cells.) By activating MAPK kinase (MEK)/ERK pathway via FGF receptors, undifferentiated state of stem cell could be maintained. (Vallier L et al., 2005, J Cell Sci.) However, since bFGF (basic FGF) is vulnerable to heat, Lotz S et al (2013, Plos one) reported that upholding pluripotency during cell growth is a serious issue. The considerable amount of cell would experience undesirable and stochastic differentiation, which cannot be detected by average-based analysis tool, such as immune-blotting. This might affect to further experiment or therapeutic treatment.

Thus, the better understanding about FGFR-ERK cascade in single-cell-level is needed to control cell fate in proper manner.

In the previous collaborative research, we found that sustained stimulation of epidermal growth factor (EGF) or neuronal growth factor (NGF) induce heterogeneous ERK kinetics in single-cell-level of PC-12 cells, which is well-established differentiation model, by temporally controlled bFGF stimulation. This could not be captured by classic experimental method, such as immune-blotting. Furthermore, we observed that ERK activity can be retriggered with pulsatile stimulation of EGF or NGF, giving synchronized cell response throughout the population. Using this, we set up the novel mathematical model, and based on that, we could re-wire the cell fate.

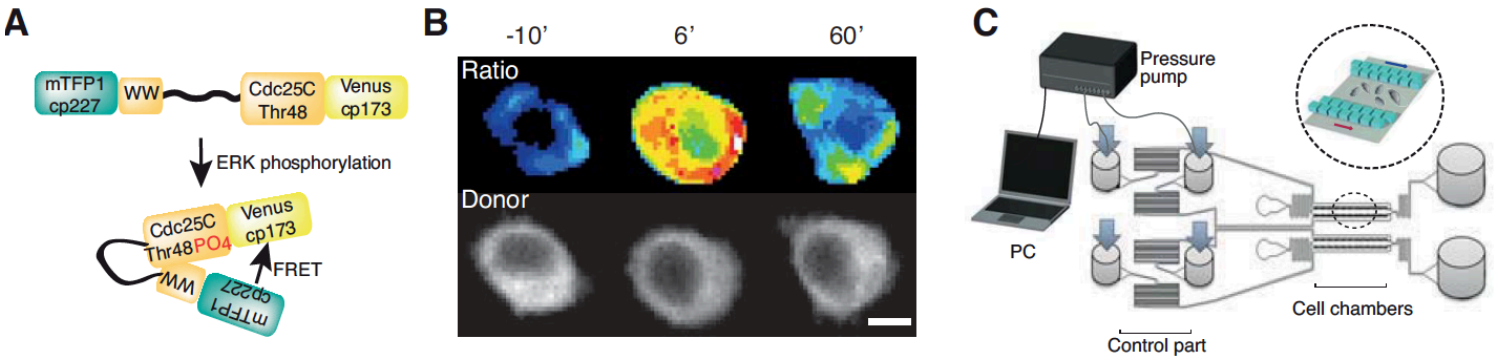

Figure 1. (A) EKAR2G FRET biosensor. (B) Ratiometric and mTFP1 donor images of EKAR2G in an EGF-stimulated PC-12 cell at the indicated time points, with t=0' corresponding to EGF application. Scale bar = 20 μm. (C) Flow-based, microfluidic device for temporal GF delivery. Computer-controlled, pressure pump enables mixing of medium and GFs in the control part

This research is initiated by the phenomena. (1) By FRET biosensor and microfluidics, and long-term live-cell imaging, we would understand the kinetics of ERK in single-cell-level, by dynamic stimulation with bFGF. (2) Using the result, we would set the strategy to "retrigger" ERK activity in a closed-loop system, leading to designated cell response. Here, we have tried to understand the ERK response of PC-12 based on the idea we had from previous collaborative research.

3) Description of the main results obtained

Result 1. ERK response by sustained FGF stimulation.

Since sustained GF stimulation is correlated to the classical dish-based culture method, we examined sustained FGF stimulation to EKAR PC-12 system. To compare the kinetics, we tested EGF 25ng/ml, NGF 50ng/ml, and FGF 25ng/ml. It is well known in PC-12 cell that EGF gives transient response, inducing proliferation, while NGF gives sustained response, leading differentiation. In Figure 2, the average response by each GF is plotted. EGF and NGF were shown to be transient and sustained, respectively. In FGF stimulation, the first peak was weaker then both GF, and was shown sustained kinetics.

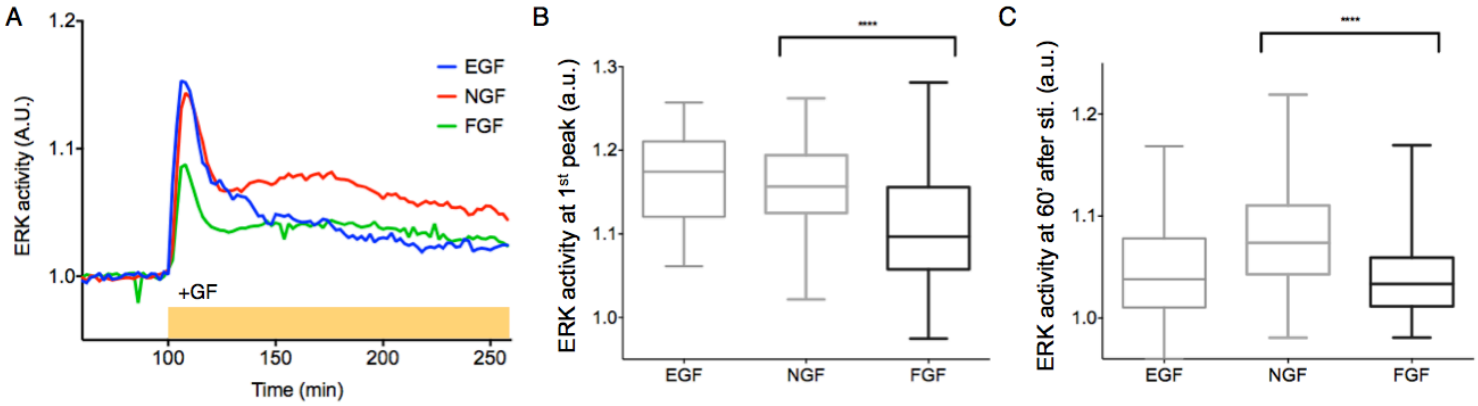

Figure 2. (A) The average ERK response from sustained stimulation of EGF 25ng/ml, NGF 50ng/ml and FGF 25ng/ml. Comparisons of (B) ERK activity at the first peak and (C) 60' after stimulation.

One interesting characteristic of FGF pathway is the biphasic nature of the cellular response. In contrast to EGF or NGF, high level of FGF2 is known to have weaker response than intermediate concentration. Recently, J.Kanodia et al (2014, Cell comm.& sig.) explained this phenomena using computational and experimental approach. They concluded that the competition between binding of the ligand FGF 2 to HSGAG and FGF receptor leads to biphasic response.

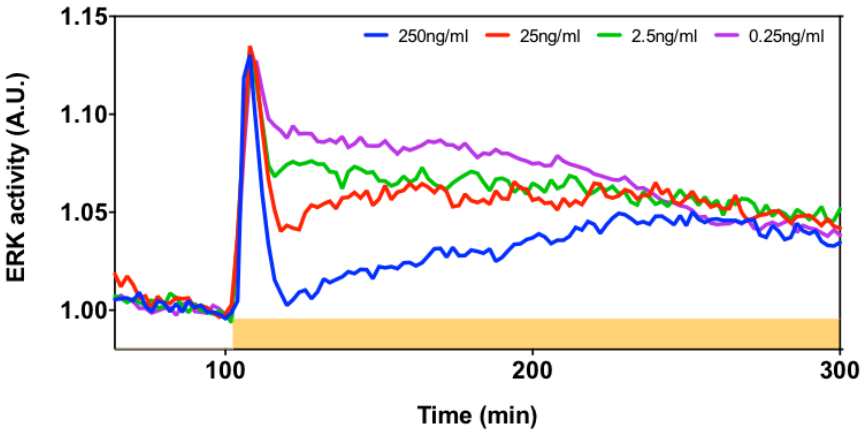

Figure 3. The average ERK response by sustained stimulation with the different concentration of FGF.

To identify this "biphasic" response, we stimulate the cell with different concentration of FGF2. As shown in Figure 3, the higher level of bFGF induced the faster adaptation kinetics, in average.

In single-cell-level, we compare the relative ERK activity, 60min after the stimulation. (We call this as "latency.") In 250ng/ml bFGF stimulation, cells were shown to have narrower latency distribution

to others. This might indicate that the strength of negative feedback to ERK activity is proportional to the amount of FGF.

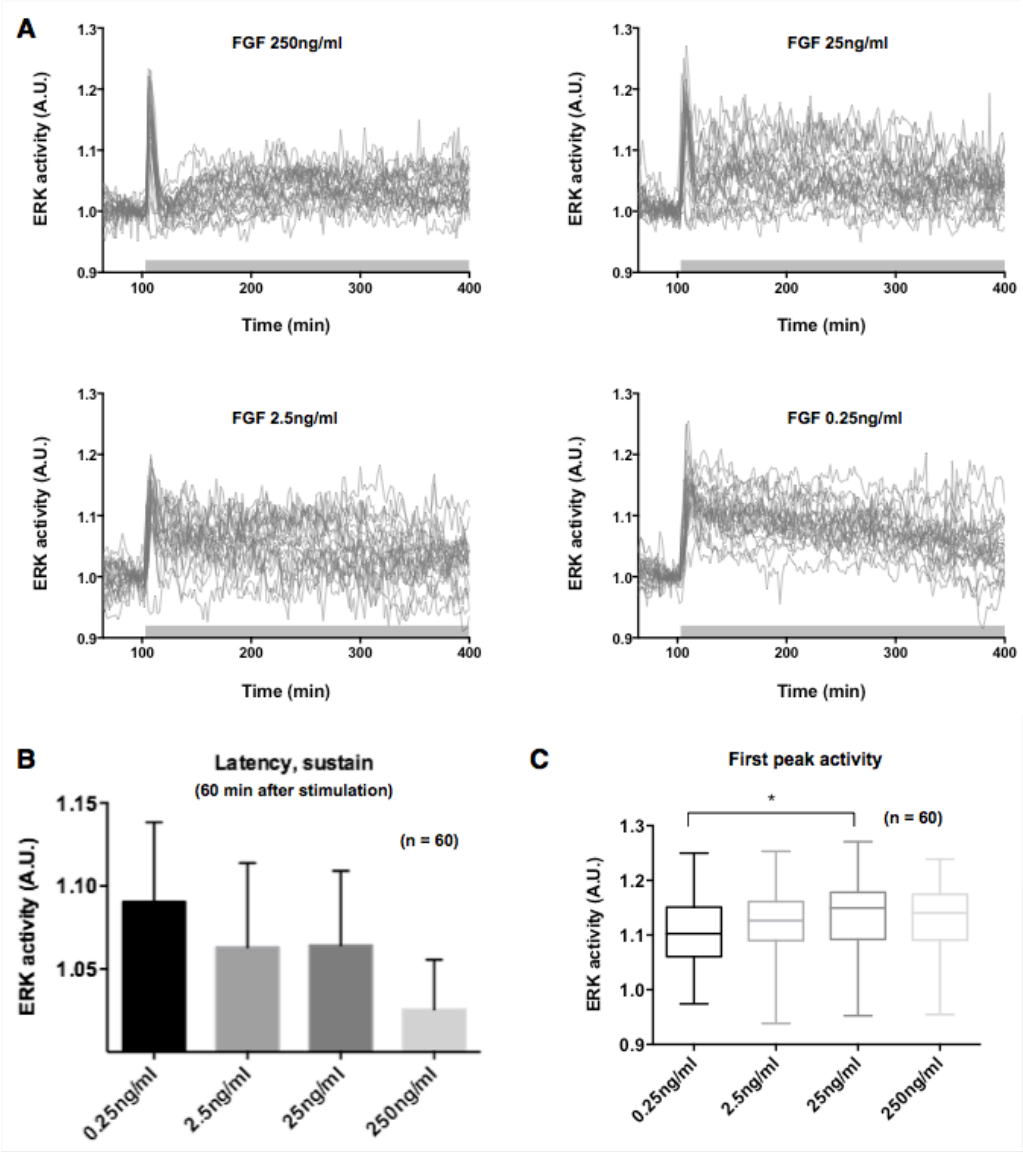

Figure 4. (A) ERK trajectories from the different FGF sustained stimulation, in single-cell-level. Comparisons of (B) latency and (C) first peak amplitude in each ERK response.

Result 2. ERK response by pulsatile FGF stimulation.

Previous experience about EGF or NGF reports that retriggering characteristic of ERK response to pulsatile GF stimulation is the key to induce designated ERK profile to lead designated cell fate determination. Thus, we observed ERK response to temporally controlled FGF stimulation.

In contrast to sustained stimulation, the higher concentration of bFGF pulse could induce the higher latency. As shown in Figure 5, stimulation of 250ng/ml bFGF in 3 min provoke sustained ERK activity, while the one of 0.25ng/ml give transient kinetics. We also stimulate cells with a longer time of pulse,

5min, 10min, 20min, and 40min. In 250ng/ml stimulation case with longer then 10min pulse, fast adaptive behavior was observed, but ERK activity was “retriggered” after the removal of growth factor.

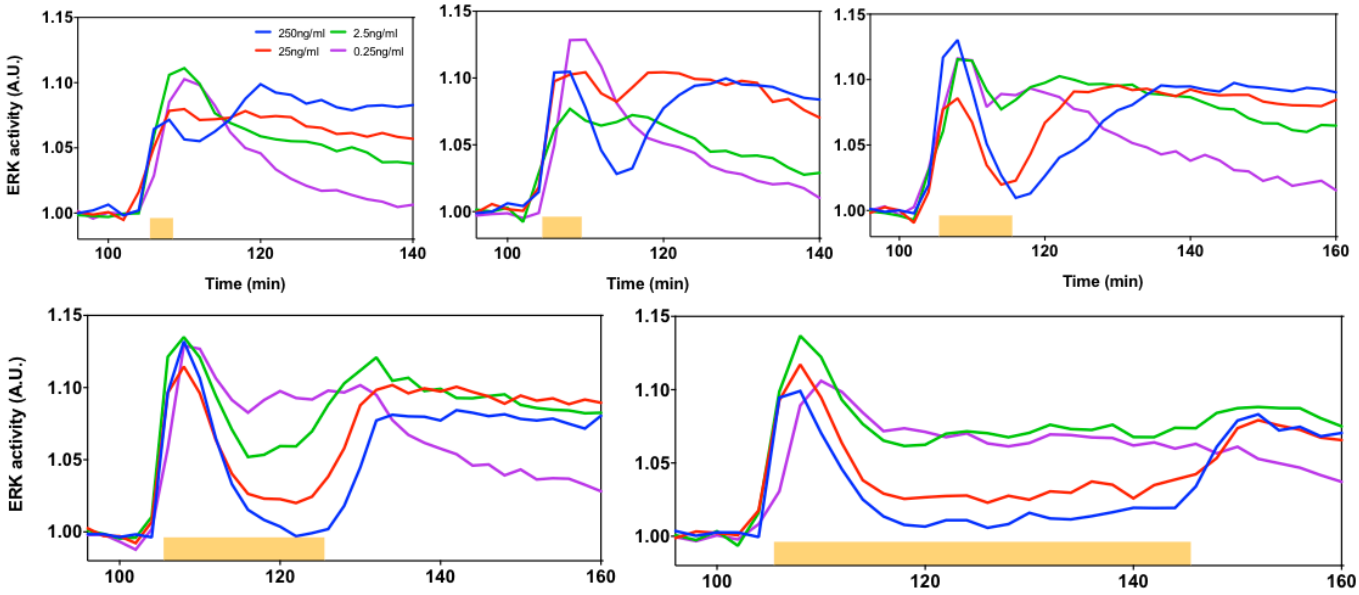

Figure 5. The average response by pulsatile FGF stimulation.

In single-cell-level, heterogeneous ERK activity was observed after ten-minute-pulse of 250ng/ml bFGF. However, 0.25ng/ml bFGF 10min pulse induces transient kinetics.

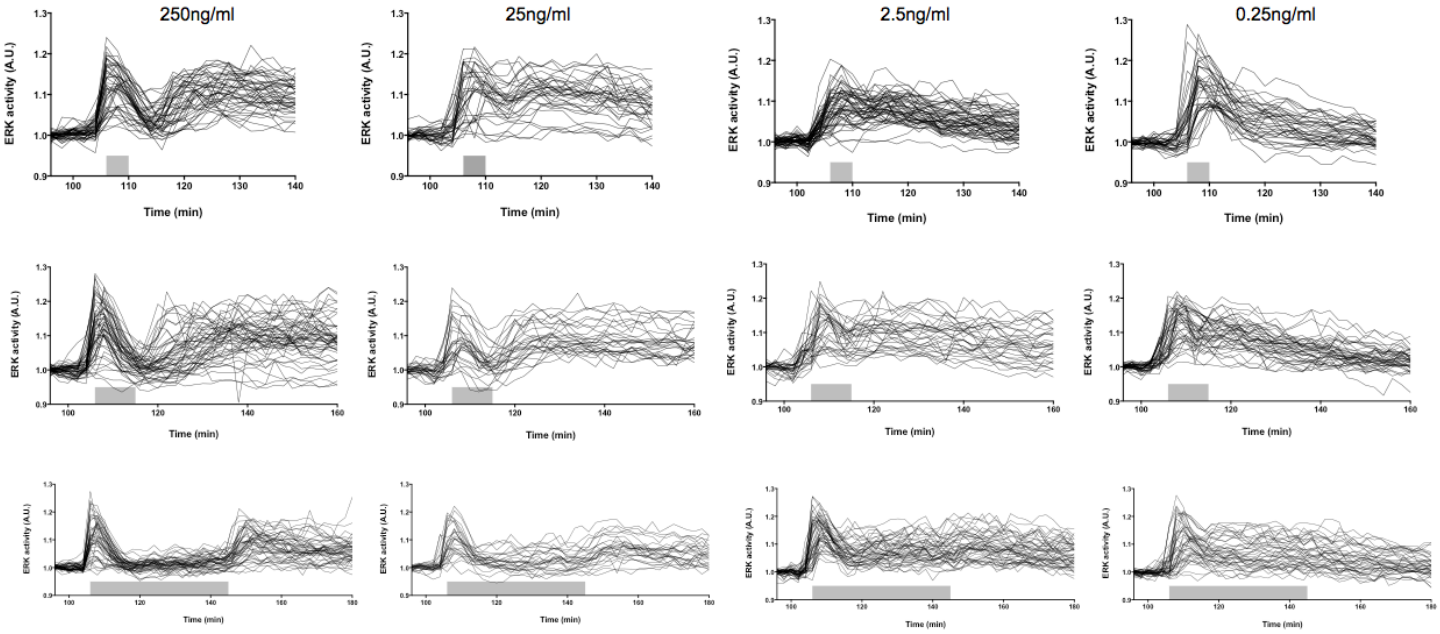

Figure 6. ERK trajectories from the different FGF pulsatile stimulation, in single-cell-level.

Result 3. ERK response by multiple pulsatile FGF stimulation.

In the previous research, ERK could be retriggered multiple times by multiple pulsatile EGF or NGF stimulation with respect to certain frequency. With this strategy, we could mimic the sustained ERK kinetics, which is the feature of NGF stimulation, using EGF multiple pulse regime. Furthermore, we could differentiate PC-12 with multi-pulse EGF, which is known as proliferative cue. This indicates that cell fate could be manipulated by modulating ERK kinetics from the dynamic extracellular stimulation.

However, FGF gives very different scheme of "retriggering," which have never been reported. As shown in Figure 6, in high frequency of stimulation, ERK could not be retriggered. The average showed the same trend as we got from sustained curve. In low frequency of stimulation, the average kinetics of ERK diverse with respect to FGF concentration. In high level of FGF, 250ng/ml, first pulse induce sustained ERK activity, but the second input induce inhibition of ERK activity. On the other hand, low concentration of FGF, 2.5ng/ml, ERK system could be retrigger multiple time, but the amplitude decrease as time goes, as we observed in EGF or NGF.

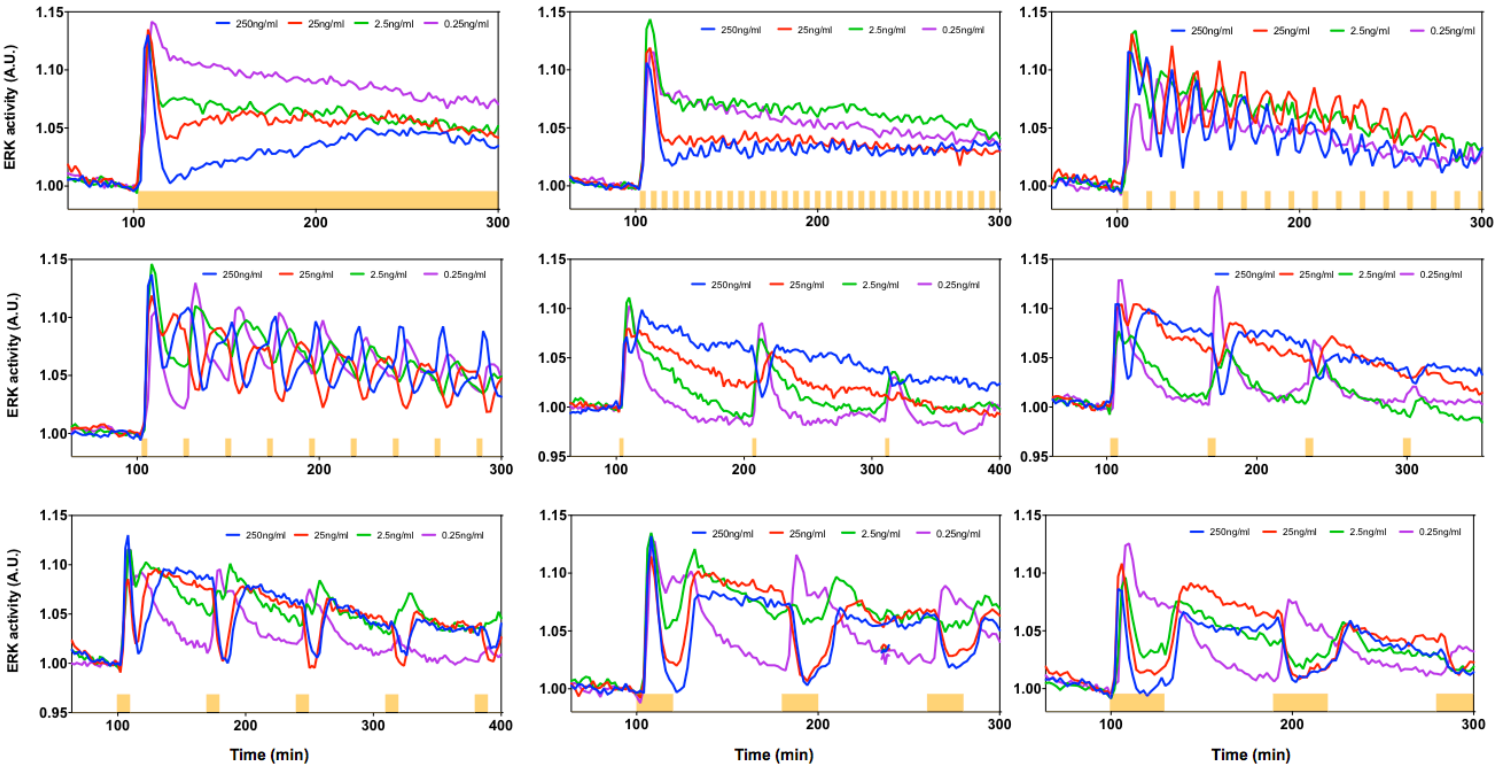

Figure 7. The average response by pulsatile FGF stimulation

In single-cell-level, heterogeneous activity induced by 250ng/ml FGF pulse deactivated simultaneously with the followed input pulse. Stimulation with low concentration FGF, 2.5ng/ml or under, could retrigger cells with heterogeneous activity distribution.

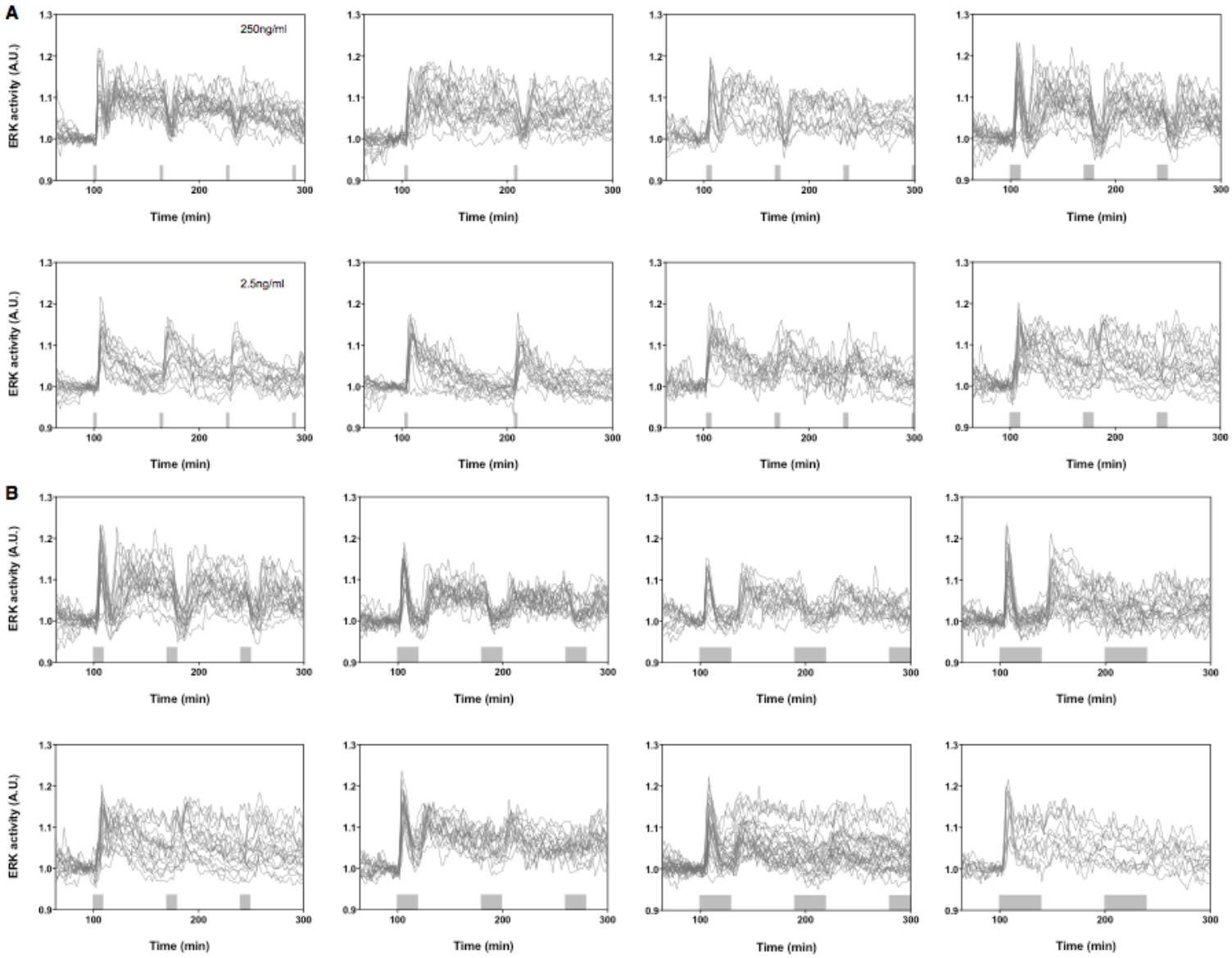

Figure 8. ERK kinetics in single-cell-level, induced by temporally controlled FGF stimulation with (A) 250ng/ml and (B) 2.5ng/ml FGF.

Summary

With Integrated platform of FRET biosensor and microfluidics, we could explore ERK kinetics by different temporal stimulation regime in single-cell-level. In this research, we focused about PC-12 response from a variety of FGF stimulation. Different from the previous experimental method, which only can stimulate in static manner, we could monitor the ERK response from pulsatile stimulation. Also, by inputting multiple pulse into the system, we could observe a novel feature related FGFR-ERK cascade.

In sustained stimulation of FGF, we could observe that the higher the concentration, the faster the adaptation of the system. In 250ng/ml stimulation, response was transient throughout the population, while 2.5ng/ml or lower amount induces sustained kinetics with heterogeneous distribution. In a single

pulse regime, on the contrary, high concentration of FGF induce heterogeneous and bistable ERK activity, while low concentration induce transient ERK kinetics throughout the population.

Stimulation pattern / Concentration	Static (sustained)	Pulsatile (First pulse)	Pulsatile (Second pulse)
High FGF concentra	Transient	Sustained, heteroger	Deactivate ERK
Low FGF concentra	Sustained, heterog	Transient	Retrigger ERK

Table 1. Rational of ERK response from FGF stimulation regime.

In multiple pulse stimulation case, the second pulse of high concentration FGF could deactivate the residual ERK activity induced by the first pulse. In contrast, low concentration of FGF could retrigger the ERK activity by multiple times.

FGF is key element to understand cell fate decision in variety of cell type, especially in stem cell. In collaboration with Khammash group in ETH, we are now rewiring the cascade with known element. Based on real-time activity of ERK by temporally controlled stimulation, we expect to uncover the hidden characteristics of ERK signaling pathway. After we have hypothetical mathematical model, we are going to perform perturbation experiment to prove the model. Our novel method will reveal the novel feature of ERK signaling pathway that is significant to stem cell manipulation.

4) Future collaboration with host institution (If applicable)

Based on the single-cell-level result on a variety of FGF stimulation pattern, we are now trying to set up a mathematical system model to describe. In collaboration with Jan Mikelson (Khammash group in D-BSSE, ETH), we expect to rewire featured elements in FGFR-ERK signaling cascade. Furthermore, to proof-of-concept, we are planning to perform the perturbation experiments based on the new model.

After we get model, we will set the strategy to control ERK response in closed-loop system, with FGF. It has its advantages since FGF was observed to have more dynamic characteristics to ERK activity than EGF or NGF. Our novel method will reveal a new insight for ERK signaling pathway that is significant to stem cell manipulation.

5) Projected publications/articles resulting or to result from the grant (ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)

Hyunryul Ryu, Jan Mikelson, Yannick Blum, Noo Li Jeon, Mustafa Khammash, and Olivier Pertz, "Investigation of FGFR-ERK signaling cascade by temporally controlled bFGF stimulation," *in preparation*.

6) Other comments (If any)

승객정보(Traveller)

승객명(Passenger Name)	**RYU/HYUNRYULMR**
예약번호(Booking Reference)	P65KBA / 1G
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여정 (Itinerary)

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루프트한자 독일항공 **예약상태(Status)** OK

출발(Departure)	**서울, Incheon Intl Arpt (ICN)**	**01JUN15**	**12:15**	
도착(Arrival)	뮌헨, Munich Intl Arpt (MUC)	01JUN15	16:50	Terminal No : 2

운임(Fare Basis)	KHRCKR	유효기간 Not Valid Before	01JUN15
수하물(Baggage Allowance)	1Piece(s)	Not Valid After	01JUN15

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출발(Departure)	**뮌헨, Munich Intl Arpt (MUC)**	**01JUN15**	**18:35**	Terminal No : 2
도착(Arrival)	바젤, Basel EuroAirport Swiss (BSL)	01JUN15	19:30	

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출발(Departure)	**바젤, Basel EuroAirport Swiss (BSL)**	**31OCT15**	**14:55**	
도착(Arrival)	프랑크푸르트, Frankfurt Intl (FRA)	31OCT15	15:55	Terminal No : 1

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수하물(Baggage Allowance)	1Piece(s)	Not Valid After	31OCT15
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루프트한자 독일항공 **예약상태(Status)** OK

출발(Departure)	**프랑크푸르트, Frankfurt Intl (FRA)**	**31OCT15**	**18:00**	Terminal No : 1
도착(Arrival)	서울, Incheon Intl Arpt (ICN)	01NOV15	12:05	

운임(Fare Basis)	KHRCKR	유효기간 Not Valid Before	31OCT15
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