Src Activity Dynamic Detection In The Initiation Of Tension-released Cell Migration

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Introduction: Cell migration is critical for embryonic development, wound healing and cancer metastasis. Protein tyrosine kinase Src plays important roles in cell migration and thus it is important to understand how molecular signals of Src regulate cellular migration and function. Here we present a live cell imaging and analysis system to observe and quantify the coupling between molecular activities and the initiation of migration. This system combines three technologies, including micro-pattern, genetic encoded fluorescent biosensor based on fluorescence resonant energy transfer (FRET) and automated analysis. This system allowed the detection of down-regulation of Src activity coupled with the membrane protrusion at cell front when migration was initiated.

Materials and Methods: Using soft-lithography method, a micro-pattern is imprinted on a thin PDMS gel membrane (10 μm in depth) to allow the cells be seeded in micro-sized wells. During imaging, the PDMS membrane is peeled off to enable the cell migration (Figure 1A). The surface of the glass bottom dish is coated with Fibronectin (10 μg/ml). The HUVEC cells are infected with the FRET-based Src biosensor (Figure 1B) in adenovirus 48 hours before imaging [1-2]. The FRET ratio images are calculated and the boundary evolution is tracked with a level-set method [3] programmed in MATLAB (Figure 1C).

Results and Discussion: The image analysis results are shown as in Figures 1D-1L. The sampled ECFP/YPet emission ratio maps represent the variation of Src activity along spatial and temporal dimensions (Figure 1D). The x-axis represents the initial boundary length (μm) and the y-axis represents time (min). After peeling-off the micro-pattern, the migration regions (mostly located on the frontier of the polarized HUVEC cell) showed low Src activity, while the Src activity at the non-migrations of the same cell remained high. Two representative time courses (M: migration region; NM: non-migration region) are shown in Figure 1E. A statistical comparison of normalized Src activity among cells is shown in Figure 1F (PO: peel-off the micro-pattern).

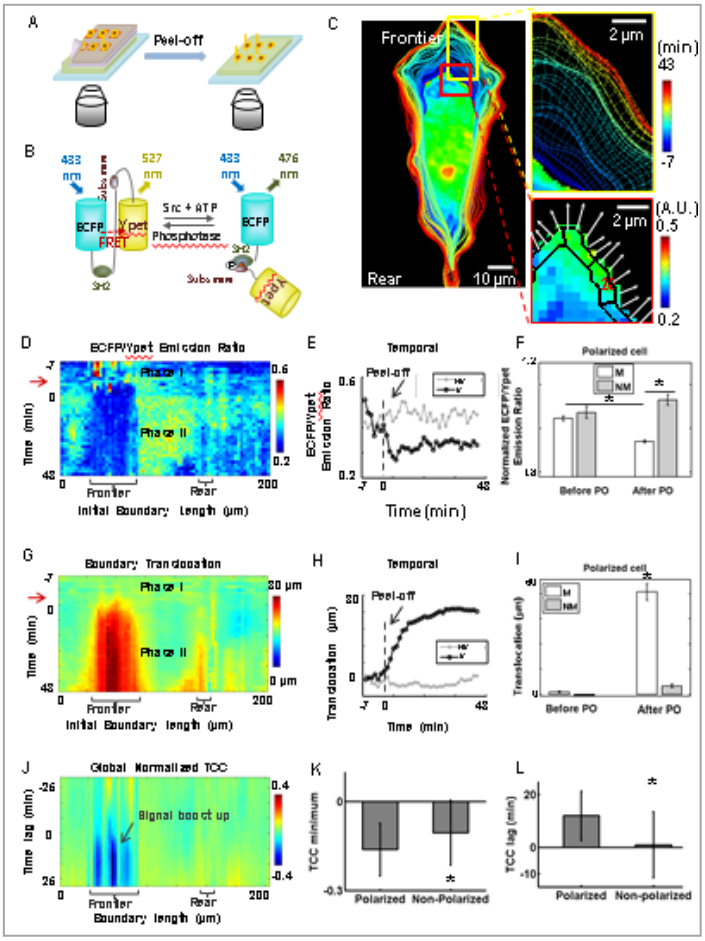

Figure 1. HUVEC cell migration initiation detection

Interestingly, in temporal dimension, the Src activity decreases significantly after peeling-off the micro-pattern within migration regions. In spatial dimension, Src activity in the migration region is significantly lower than the non-migration region after peeling-off. Figures 1G-1I show the translocation of the cell membrane boundary, which demonstrates an opposite trend from the Src activity map in Fig. 1D-1F. The temporal cross-correlation map between Src activity and cell boundary translocation is calculated as shown in Figure 1J. In the migration region, the temporal cross-correlation course shows the lowest value (blue color) at a positive time lag, which means that the Src activity decreased before cell membrane protrusion. The statistical comparison of the temporal cross-correlation (Figure 1K) and time lag (Figure 1L) display that the difference between the migration and non-migration regions is significant.

Conclusions: We developed an advanced imaging and analysis system combining the micro-pattern, biosensor, and analysis tools to detect the spatiotemporal dynamics of molecular signal at the initiation of cell migration. The results show that the down-regulation of Src activity is coordinated with the HUVEC cell membrane protrusion.

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