Integrating live-cell fluorescent microscopy and signal processing to discover the relationship of invadopodia digging cycles with extracellular matrix crosslinking ratio.

Kamyar Esmaeili Pourfarhangi[1], Aviv Bergman[2,3], Bojana Gligorijevic[1,4]

[1]Bioengineering Department, College of Engineering, Temple University, Philadelphia, PA, USA
[2]Systems & Computational Biology Department, Albert Einstein College of Medicine, Bronx, NY, USA
[3] Santa Fe Institute, Santa Fe, NM, USA
[4]Cancer Biology Program, Fox Chase Cancer Center, PA, USA

Kamyar.esmaeili@temple.edu, Abergman@gmail.com, Bojana.gligorijevic@temple.edu

While the study of many processes happening in a single living cell has become easier than ever via live-cell fluorescent microscopy, the dynamic nature of such processes has highlighted the importance of signal processing.

Cancer, which is known as the leading cause of death in western world, initiates with formation of a tumor and spreading of tumor cells, in a process called metastasis. During the metastasis, cancer cells enter the blood circulation, travel to distant organs where they can form the secondary tumors. In order to enter the blood vessels, cancer cells assemble invadopodia, protrusions which enzymatically and mechanically degrade extracellular matrix (ECM) around blood vessels [1].

It has been shown that an actively degrading invadopodium exhibits cycles of extension and retraction, which we refer to as "digging cycles" [2]. Further, recent work reported that the crosslinking ratio of ECM in vivo plays an important role in invadopodia activity [1]. While the digging cycles of invadopodia are linked to their degrading function [3,4], the direct relationship between the dynamics of these cycles and the crosslinking ratio of the surrounding ECM is unknown.

In this study, we investigated the influence of ECM crosslinking level on the frequency of invadopodia digging cycles and degradation. First, invadopodia brightness was monitored via time-lapse fluorescent microscopy in breast cancer carcinoma cell line (MTLn3) cultured on gelatin at different crosslinking ratios. Gelatin was crosslinked by glutaraldehyde (GTA) at various concentrations (0.01, 0.05, and 0.2% v/v GTA). Next, the time-lapse movies were processed via imajeJ in order to record the oscillations in invadopodia brightness stemming from the digging cycles of invadopodia. Oscillations were then filtered via Fourier Transform by cutting off the high frequencies attributed to the CCD camera noise. Further, we applied autocorrelation on the filtered oscillations to extract the periodicity of the digging cycles. Quantifications were performed for oscillations detected from cells cultured on all the three crosslinking ratios. At this stage, we observed that invadopodia digging cycles frequency has a biphasic trend in response to the increase in gelatin crosslinking ratio (the maximum frequency 3.07 mHz at 0.05% GTA).

Next, we investigated the invadopodia degradation at each crosslinking ratio. Interestingly, results showed that as gelatin crosslinking level increases, changes in the amount of invadopodia degradation perfectly correlate with that of the frequency of the digging cycles. This result suggests that the invadopodia digging cycles and ECM degradation are highly coordinated cell activities.

1. Gligorijevic, B., et al.. "Multiparametric classification links tumor microenvironments with tumor cell phenotype."*PLoS Biol* 12.11 (2014): e1001995.
2. Magalhaes, M., et al. "Cortactin phosphorylation regulates cell invasion through a pH-dependent pathway." *The Journal of cell biology* 195.5 (2011): 903-920.
3. Labernadie, A., et al. "Dynamics of podosome stiffness revealed by atomic force microscopy." *Proceedings of the National Academy of Sciences* 107.49 (2010): 21016-21021.
4. Van den Dries, K., et al. "Interplay between myosin IIA-mediated contractility and actin network integrity orchestrates podosome composition and oscillations." *Nature communications* 4 (2013): 1412.

Integrating live-cell fluorescent microscopy and signal processing to discover the relationship of invadopodia digging cycles with extracellular matrix crosslinking ratio

Kamyar Esmaeili Pourfarhangi[1], Aviv Bergman[2], Bojana Gligorijevic[1,3]

[1]Bioengineering Department, College of Engineering, Temple University, Philadelphia, PA, USA

[2]Systems & Computational Biology Department, Albert Einstein College of Medicine, New York, NY, USA

[3]Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA, USA

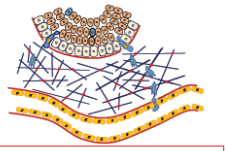
Abstract

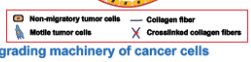
- Metastasis is a process through which tumor cells spread throughout the patient's body.
- Invadopodia are cellular protrusions with which cancer cells degrade their immediate extracellular matrix (ECM) paving the way to enter the blood vessels and perform metastasis.
- ECM crosslinking ratio *in-vivo* plays an important role in invadopodia activity [1]
- An active invadopodium shows cycles of retractions and protrusions [2] which we refer to as "digging cycles."
- In this study, we utilize live-cell microscopy and signal processing techniques to investigate the relationship between ECM crosslinking, invadopodia digging cycles frequency, and invadopodia degradation.
- At some intermediate level of crosslinking, where invadopodia degradation shows a maximum level, digging cycles frequency shows its maximum value implying that invadopodia degradation and digging cycles are highly correlated activities.

Background

Intravasation, the onset of Metastasis

- After tumor growth, a limited number of tumor cells undergo mutations, as a result of which they become motile.
- Motile tumor cells show various types of migration, one of which is MMP-dependent adhesive migration.
- In this type of migration cells degrade the ECM via invadopodia.





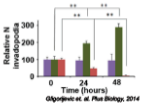
Invadopodia, the degrading machinery of cancer cells

- Invadopodia are dynamic membrane protrusions involved in the invasive motility of cancer cells. These protrusions degrade the tumor associated ECM via proteolysis.

MTLn3 cells plated on a green fluorescent gelatin, fixed after 16 h and immunostained for actin and cortactin proteins. Scale bar shows 20 μm.

ECM crosslinking level and invadopodia

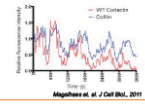
- It has been shown in vivo [1] that the increase in ECM crosslinking ratio by 1 g/kg/day L-ribose (green bars) significantly increases the number of invadopodia.
- Decreasing ECM crosslinking ratio using 6 mg/kg/day BAPN (orange bars) decreases the number of invadopodia drastically.



Gligorijevic et. al. *Plos Biology*, 2014

Invadopodia digging cycles

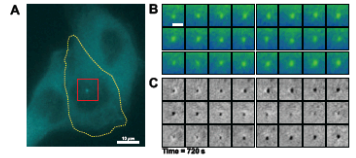
- Invadopodia digging cycles is shown in vitro [2]
- Digging cycles are suggested to be essential for invadopodia degradation



Magalhaes et. al. *J Cell Biol.*, 2011

Invadopodia digging cycles frequency detection

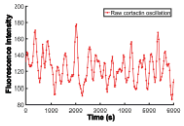
1. Time-lapse fluorescent microscopy for monitoring the dynamics of invadopodia brightness in MTLn3 breast cancer carcinoma



A. MTLn3 cells plated on gelatin. B. Dynamics of the invadopodium that is shown in panel A by the red square during 720 seconds. Scale bar shows 5 μm. C. Fitting a 2D Laplacian of Gaussian function on the images in panel B

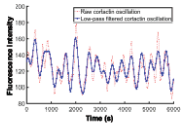
2. Quantifying cortactin oscillations

- Quantification of the fluorescence intensity of the cortactin punctum over two hours is done by ImageJ and the raw data is plotted in this figure.



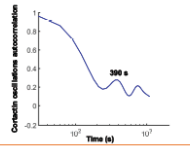
3. Applying a low-pass filter to the data via Fourier Transform

- In order to filter the noise that is recorded during image acquisition (CCD camera error) the following procedure was done:
1. Fourier transform was applied to the raw data;
2. The high frequency parts of the data was removed (>0.02 Hz).
3. Inverted Fourier transform was applied to the filtered data to restore the filtered oscillation curve.

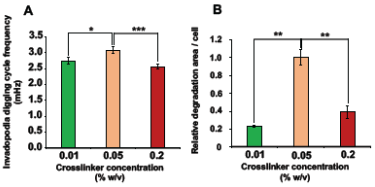


3. Applying autocorrelation to the filtered oscillations to extract the periodicity of the digging cycles

- Autocorrelation implies that in this example the digging cycle periodicity is approximately 300 seconds.
- The frequency of the digging cycles are calculated by 1/periodicity.



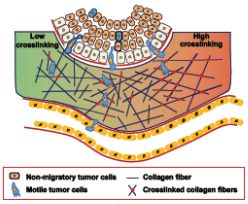
Invadopodia digging cycle frequency and degradation have a biphasic relationship with matrix crosslinking ratio



A. Bars show the invadopodia digging (cortactin oscillation) frequency in the three different matrix rigidities and represent >10 cells in 3 separate experiments per condition. B. Bar plot comparing average invadopodia degradation per cell in varying crosslinking ratios of gelatin substrate.

Conclusion

- Invadopodia digging cycle frequency and degradation show a biphasic trend as a response to ECM crosslinking ratio increase.
- Invadopodia digging cycles are indeed crucial for their degradation.
- Our data suggests that the intravasation efficiency is maximized at an intermediate level of ECM crosslinking.



Acknowledgement

We would like to thank Dr. Ved Sharma and the lab of Dr. John Condeelis for to gift of Cerulean-cortactin-MTLn3 cell line. This work was funded by NIH 5K99CA172360 and Concern Foundation award to BG and NIH R01CA164468 and R01 DA033788 to AB.