Probing Cancer Cell Mechanism Using Single Cell Atomic Force Microscope

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Introduction

- Cancer cell can get high viscosity and motility by losing cell adhesion function to metastasis
- pH value is lower than normal cell to realize as a signal for proliferation and migration

Normal cell	Cancer cell
Inside pH:6.99~7.20	Inside pH:7.12~7.65
Outside pH:7.3~7.4	Outside pH:6.2~6.9

Figure1:Normal cell and cancer cell pH value [1]

- Usually around cancer cell has poor blood circulation and the oxygen concentration gets lower
- Cancer cell inhibits function of mitochondrion and need glycolytic function
- Lactic acid is created and pH value gets lower by emission of lactic acid

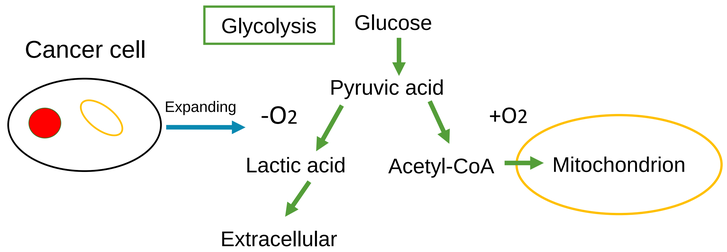


Figure2:Cancer cell cytoplasm and inside Mitochondrion [2]

Step force

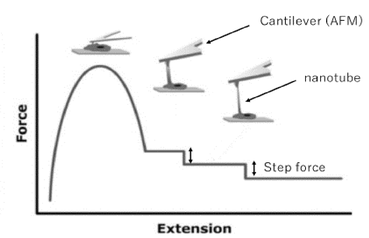


Figure3: Force vs extension of nanotube made by cell membrane [3]

- Step force is needed to break the bonding of cell membrane called nanotube
- Step force will be an indicator for cancer cell studies

Method

HCl pH:1.0 0.1mol/L

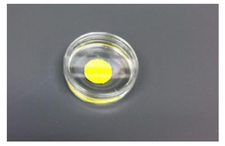
pH 6.0: add HCl 5mL

pH 6.5: add HCl 3mL

pH 7.0: normal

- Adjust the pH value of culture fluid medium by adding HCl to normal one to make HeLa cell subculture

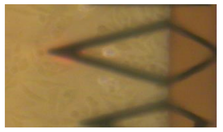
Figure4: 3 kinds of different pH medium

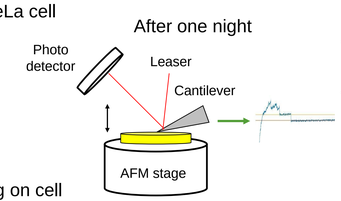


HeLa cell kept with normal medium

- Attached Hela cell on AFM substrate disc with different pH medium and keep in CO_2 incubator one night to grow it
- The concentration of CO_2 is kept 37% constantly

Figure5: Substrate for putting HeLa cell





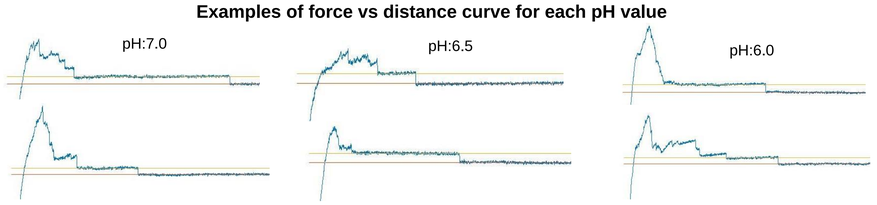
- AFM continues to attach and detach with cell
- After adjusting some values for AFM and measuring, I can get step force curves

Figure6: AFM cantilever touching on cell

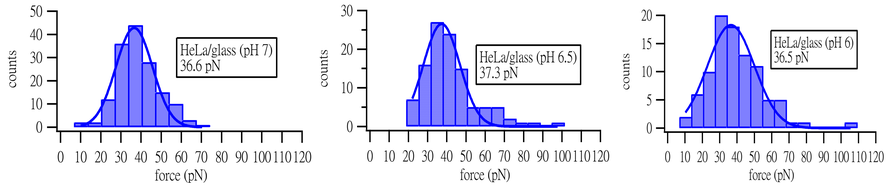
Result

Select clear and flat step force curves and make histogram

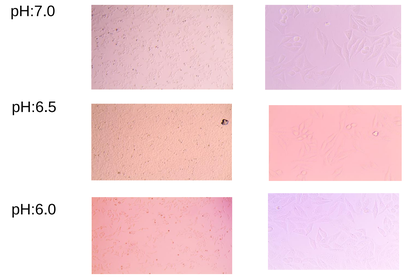
Examples of force vs distance curve for each pH value



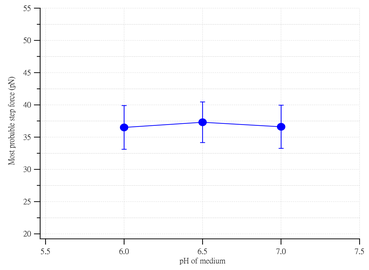
Histogram force and the number of cell with different pH value value



Optical image of HeLa cell in each pH value



Correlation between pH value and most probable force

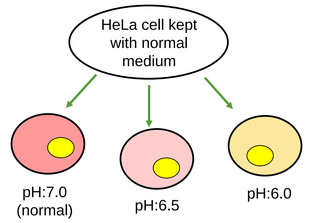


Conclusion

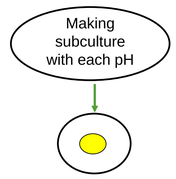
- Cancer cell properties can change depending on its microenvironment
- No major variation in forces observed
- The total number of cell which has clear and flat step force increases in this order pH 7.0,6.5,6.0

Future work

- In this time, I used three kinds pH of medium. Some pH value which is lower than pH 6.0 and higher than pH 7.0 are needed to measure
- Comparison of step force with normal or other cancer cell such as lung cancer cell under same situation
- Changing how to make subculture as shown below illustration



The experiment for this time



- pH is same one which used subculture
- adjust concentration of CO_2 incubator

Future work

Reference

[1] Cardone, Rosa A,Casavola, Valeria,Reshkin, Stephan J. 'The role of disturbed pH dynamics and the Na^+/H^+ exchanger in metastasis'(2005)
[2] Rob A. Cairns, Isaac S. Harris,Tak W. Mak,' Regulation of cancer cell metabolism'(2011)
[3] Mingzhai Sun,John S. Graham,y Balazs Hegedus,z Franc xoise Marga,Ying Zhang,Gabor Forgacs,Michel Grandboisy,' Multiple Membrane Tethers Probed by Atomic Force Microscopy'(2005)

Acknowledgements

This research was conducted as a part of 2018 Nakatani RIES program supported by Nakatani Foundation. If you want to see more information, please check this website https://www.nakatani-foundation.jp/business/ries/
Special thanks to Prof. Ching-Hwa Kiang and Tsung-Cheng Lin for excellent research guideline. Prof.Kono, Sarah Phillips and Kenji Ogawa made this program and facilitated Kiang thanks to the Welch Foundation and the Hamill innovations award for support