A Quantitative Study of Nocodazole’S Effect on HeLa Cells’ Growth Rate and F-actin Structure

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1. Introduction

HeLa cells – the first continuous cancer cell line - were isolated from human cervical cancer, which has been widely used as a research model to study mammalian cell functions. Almost every mammalian cellular process has been tested in HeLa cells [1, 2, 3].

Nocodazole, a benzimidazole derivative, was initially developed as a potential anticancer drug [4]. The drug became a popular treatment for cancer due to its ability to prevent mitosis. Besides its medical application, nocodazole has also been widely used as a biomedical research tool to many cellular functions. Nocodazole inhibits cell growth by binding to tubulin and thus suppressing microtubule polymerization, thus leading to apoptosis in dividing cells [5]. Other studies argue that Nocodazole induces mitotic arrest due to a decrease in microtubule dynamic turnover, meaning the drug significantly hindered the elongation of microtubules [6]. This discrepancy may be resulted from different doses related function of nocodazole on mammalian cells, which has not been heavily investigated.

In this experiment, we tested the survival rate of the cultured HeLa cells by introducing different concentrations of Nocodazole. At concentration of 10 M, Nocodazole blocks microtubule polymerization and it does so by taking away α/β tubulin dimers in dictyostelium cells, thus inhibiting the growth of the dicty cells [7, 8]. However, work done by Blajeski et al showed that at 1 M, nocodazole functioned to destabilize microtubules and arrest cell cycle [9]. And work done by Vasquez et al showed that at nanomolar concentration, nocodazole was able to inhibit microtubule functions. [10]

The microtubule-actin interactions are constant and critical in cells, especially in events like cell division and cell migration [11, 12, 13]. During cell division, the structural integrity of the spindle apparatus is essential for the formation of actomyosin ring at the end of anaphase and the beginning of cytokinesis. The cell would not enter cytokinesis if the spindle apparatus is disrupted, but would not stop cytokinesis once the cleavage furrow formation starts, even if spindle apparatus is destroyed [14].

The positioning of cleavage furrow was also regulated by microtubules. During the cleavage furrow formation, actin binds to microtubules and was pulled away by the
2. Materials and Methods

HeLa cells were obtained from ATCC (American Tissue Culture Center) and cultured in DMEM media supplemented with 10% FBS and antibiotics. Cell culture related reagents, DMEM media, PBS, Trypsin, FBS, were purchased from Life Technologies. Nocodazole was purchased from Sigma.

The growth rate of heLa cells was first determined by setting four 2 cm plates and adding 2ml of media in each one of them, with nocodazole or DMSO. All four plates were incubated at 37°C. On the 1st, 3rd, 4th, and 6th day after plating, one of the 2 cm plates were taken out of the incubator and the cells were counted using a hemocytometer.

In order to study the effect of nocodazole on the growth rate of HeLa cells, HeLa cells growth rate without nocazoloe was initially determined (Fig 1). Cells growth dynamics was monitored in a period of 150 hours. Cell densities in dishes were determined every 24 hours. Cell density against time was plotted as figure 1. Data was then fit by an exponential curve: $y = 858e^{0.029x}$. The calculated growth rate was 0.029, which means cells divide in every 23.9 hours.

Table 1. Cell viability assay by MTT absorptions.

| Noc Concentrations | Absorption 540 nm | Relative Cell Viability |
|--------------------|-------------------|-------------------------|
| 0 nM               | 0.869             | 100%                    |
| 100 nM             | 0.549             | 63%                     |
| 500 nM             | 0.274             | 32%                     |
| 1000 nM            | 0.162             | 19%                     |

3. Results

3.1. The Growth Rate of HeLa Cells

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3.2. Nocodazole Inhibit the Growth of HeLa Cells

Next, we want to test how nocodazole can inhibit the growth of HeLa cells. Cells were again cultured in 2-cm dishes, nocodazole at different concentrations or DMSO were added to cells. Cell density again was determined by using hemocytometer daily. As low as 100 nM, nocodazole was able to inhibit most of the cell growth. At the same time, the 500 nM and 1 µM treatments exhibited the most growth inhibition (Figure 2). The control, the DMSO treatment, showed normal growth of HeLa cells according to the previously analyzed growth rates in figure 1.

In addition to the investigation on growth inhibition of nocodazole at different concentrations, cell viability was determined by MTT assay after cells incubated with nocodazole. HeLa cells were cultured in 2-cm dish and incubated with nocodazole at different concentrations for 48 hours. Cell viability data were shown in table 1 and figure 3. At concentration as low as 100 nM, nocodazole was able to impact cell viability.
3.3. The Effect of Nocodazole on F-Actin Structure in HeLa Cells

Next, we want to test how different concentrations of nocodazole may effect the structure and dynamics of F-actin in HeLa cells. HeLa cells were cultured in chambered slides. Nocodazole or DMSO were added to cells. After 24 hours of incubation, cells were fixed and stained to visualize the F-actin structure (Figure 4). It was consistently observed that cells treated with 1 mM nocodazole showed stronger F-actin signal compare to the control cells. Therefore, it is possible that dissolved tubulin may have a positive effect on the polymerization of F-actin filaments.
4. Discussion

After cells treated with high concentration of nocodazole, we could observe clear structure and quantity difference in F-actin in nocodazole+/- conditions. The amount of F-actin appears to be higher when cells are exposed to nocodazole, it is possible that the microtubule depolymerization triggered Rho A pathway and synthesized more actin. Since Rho A can not stabilize microtubules when nocodazole is present, more and more actin were made during the process of Rho A trying to stabilize microtubules [18].

Structurally, we could see a clear circular distribution of F-actin in cells that were exposed to nocodazole, while F-actin in control group shows no clear pattern in their distribution. Actin serves an essential role in mitosis by help spindle apparatus to orientate and bind to specific regions in the cell, to make sure that the placement of future division plane is correct. It is possible that in the nocodazole treated cells the actin spreads out in the cell and prepares to bind to microtubules during mitosis, but since microtubules were depolymerized by nocodazole, actin has nothing to binds to and stays in the circular distribution. However, in the control group, actin served their function by properly orientated microtubules and actin has moved out of this circle [16].

These clear differences in F-actin give us reason to conclude that our hypothesis, that if microtubules in cells are disassembled, we would be able to see a significant change in either the structure or the quantity of actin in the cell.

However, we are still uncertain of what caused these changes, and a lot of mechanisms involved in these changes remain unknown. Future research can be focused on looking for the exact mechanisms that caused these changes.

Regarding our first experiment of growth rate of the HeLa cells, both the group and class results supported our hypothesis that doubling time of the cells would be around 24 hours. At concentration as low as 100 nM, nocodazole exhibited strong inhibitory effect on cell growth. Since it was shown that nocodazole disrupts microtubule at concentration
higher than 1 μM, it is reasonable for us to contemplate that how does nocodazole exert the inhibitory effect at the concentration of 100 nM. An alternative mechanism of nocodazole inhibit cell division may exist.

For the survival test of our HeLa cells when treated with Nocodazole, cell density counting revealed interesting data that supported our hypothesis. As expected, the 1 μM of nocodazole treatment exhibited the least cell density at the end of the treatment, followed by the 500 nM (Figure 3). These two results show that both of these Nocodazole concentrations were effective in reducing the HeLa cell populations. The control treatment (DMSO) exhibited a cell density that was expected according to the previously analyzed growth rate of our HeLa cells in regular conditions. The 100 nM Nocodazole treatment group showed the highest post-treatment value of cell density (Figure 3) and thus the most growth over the course of the experiment, supporting our hypothesis. This finding can be related to Darwinian Evolution as these cells were treated with enough Nocodazole to develop adaptions that allowed the cells to thrive when exposed to Nocodazole and as the treatment concentration was not effective nor lethal, it allowed the cells that developed adaptions, the “most fit” cells in Darwinian language [19], to proliferate and thus pass on their resistance against Nocodazole to the newly generated cells.

In the future experiment, nocodazole at concentration of 10 nM and 50 nM will be tested as well to see how effective these concentration may inhibit cell growth. However, in a shorter incubation period, 100 nM of nocodazole showed 30% inhibitory effect on cell viability. All together, these results suggest cells may not able to metabolize nocodazole effectively. At low concentration, the drug effect may last for 4-5 days. It also suggests that in terms of treatment, low concentration in combination with long treatment period maybe an option.

The doubling time of HeLa cells was measured to be 23.9 hours. The previous study done by Meck et al [20] found that the doubling time of HeLa cells in vitro was about 28.8 hours. The result obtained in this experiment was close to but not exactly matches with Meck’s result. There are several possible reasons for the discrepancy. The HeLa cells have been cultured extensively, therefore HeLa cells from different lab or batch may have different features since they are at high risk of mutation. Secondly, the culturing condition maybe slightly different between lab to lab. Overall, knowing the growth rate is helpful, so that researchers could potentially use this number to assess their cell line’s growth condition.

It will also be interesting to assess nocodazole’s drug effect on other cancer cells. Nocodazole, although it has been used as an anti-cancer drug for a long time, is still used in some cases clinically. Determine the effective concentration and treatment period will be very helpful in determine the drug amount and treatment period clinically. Our results suggest that a low dose but longer treatment combination maybe considered to use nocodazole to have negative impact on cancer cell proliferation.

In summary, this series of experiments with HeLa cells serves as an example of how effective these cells are for culturing, treatment exposure, and characterization in vitro. There were some limitations to our experiments such as the short periods of observation of growth short, or the fact that we only experimented with three different concentrations of Nocodazole. For future studies with HeLa cells we recommend larger observation periods and replications of such in order to accurately monitor the growth rate of these cells. For studies that also aim at observing Nocodazole’s effect on cell structures we recommend testing with more concentrations as well as performing in vivo experiments in which Nocodazole was introduced to cancerous cells in a mouse model, if plausible this experiment could give more insight into Nocodazole’s ability to disrupt the F-actin filaments in vivo.

5. Conclusion

In conclusion, our results indicate that nocodazole was able to inhibit cell growth in a nanomolar range concentration. At micromolar concentration, the disturbance of microtubule clearly has an impact on the F-actin dynamics and structure.

References

[1] Masters JR. Nat Rev Cancer. 2002 Apr;2(4):315-9. Review.
[2] Beskow LM. Annu Rev Genomics Hum Genet. 2016 Aug 31;17:395-417. doi: 10.1146/annurev-genom-083115-022536. Epub 2016 Mar 3. Review.
[3] AccessScience Editors. (2014). Importance of HeLa cells. In AccessScience. McGraw-Hill Education.
[4] Blajeski, A. L., Phan, V. A., Kottke, T. J., & Kaufmann, S. H. (2002). G1 and G2cell-cycle arrest following microtubule depolymerization in human breast cancer cells. The Journal of Clinical Investigation, 110(1), 91–99.
[5] Jordan, M. A., Thrower, D., & Wilson, L. (1992). Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. J cell Sci, 102(3), 401-416.
[6] Vasquez, R. J., Howell, B., Yvon, A. M., Wadsworth, P., & Cassimeris, L. (1997). Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. Molecular biology of the cell, 8(6), 973-985.
[7] Ngo T, Miao X, Robinson DN, Zhou QQ. Acta Pharmacol Sin. 2016 Nov;37(11):1449-1457. doi: 10.1038/aps.2016.57. Epub 2016 Aug 29.
[8] Zhou Q, Kee YS, Poirier CC, Jelinek C, Osborne J, Divi S, Sureel A, Will ME, Eggert US, Müller-Taubenberger A, Iglesias PA, Cotter RJ, Robinson DN. Curr Biol. 2010 Nov 9;20(21):1881-9. doi: 10.1016/j.cub.2010.09.048. Epub 2010 Oct 14.
[9] April L, Blajeski, Vy A, Phan, Timothy J, Kottke, Scott H, Kaufmann, J Clin Invest. 2002 Jul 1; 110(1): 91–99. doi: 10.1172/JCI13275.
[10] Vasquez RJ, Howell B, Yvon AM, Wadsworth P, Cassimeris L., Mol Biol Cell. 1997 Jun;8(6):973-85.

[11] Poulter NS, Thomas SG. Int J Biochem Cell Biol. 2015 Sep;66:69-74. doi: 10.1016/j.biocel.2015.07.008. Epub 2015 Jul 23. Review.

[12] Akhshi TK, Wernike D, Piekny A. Cytoskeleton (Hoboken). 2014 Jan;71(1):1-23. doi: 10.1002/cm.21150. Epub 2013 Oct 29. Review.

[13] Cabrales Fontela Y, Kadavath H, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Nat Commun. 2017 Dec 7;8(1):1981. doi: 10.1038/s41467-017-02230-8.

[14] HIRAMOTO Y. J Cell Biol. 1965 Apr;25:SUPPL:161-7.

[15] Sider, J. R., Mandato, C. A., Weber, K. L., Zandy, A. J., Beach, D., Finst, R. J., Bement, W. M. (1999). Journal of Cell Science, 112(12), 1947.

[16] Lutz, D. A., Hamaguchi, Y. & Inoue, S. (1988). Cell Motil. Cytoskeleton 11, 83–96.

[17] Forscher, P., Bement, W. M., Waterman-Storer, C. M., Mandato, C. A., Schaefer, A. W., & Rodriguez, O. C. (2003). Nature Cell Biology, 5(7), 599-609.

[18] Palazzo, A. F., Cook, T. A., Alberts, A. S. & Gundersen, G. G. (2001). Nature Cell Biol. 3, 723–729.

[19] Peitzsch, C., Tyutyunnykova, A., Pantel, K., & Dubrovska, A. (2017, February). In Seminars in Cancer Biology. Academic Press.

[20] Meck RA, Carsten AL, Kelsch JJ. Cancer Res. 1976 Jul; 36(7 PT 1):2317-20.