Examining the Tumorigenic Ability of Cytokeratin 8/18 Filaments in Cervical Cancer Cells In vitro
Stephanie Parisi, David H. Townson PhD
Department of Molecular, Cellular, and Biomedical Sciences; University of New Hampshire

Abstract
Cervical cancer is the second-leading cause of cancer-related death among women. Infection of the cervix by the human papillomavirus (HPV) often leads to cervical cancer. However, the cellular mechanisms by which HPV-infected cells proliferate, migrate, and evade immune attack during metastasis are relatively unknown. We have determined that certain cervical cancer cells (i.e., HeLa cells), not only contain HPV but also have two distinct characteristics: some cells readily express cytokeratin 8/18 filaments (K+ cells), while others do not (K- cells). The question posed in this study is: What is the rate of proliferation and motility between K+ and K- HeLa cells, and is the K+ cell more aggressive in its growth? Accordingly, growth experiments over seven days of culture indicated the K+ cells multiplied faster than the K- cells (0.67 ± 0.06 vs. 0.40 ± 0.03 doublings per day, respectively), and had a shorter generation time 1.52 ± 0.15 vs. 2.52 ± 0.18 days, n = 3 experiments). A wound healing assay was conducted that showed the K+ cells migrate faster than controls vs. the K- cells. The rate of migration of K+ vs. K- cells was 4.88 ± 0.01 to 4.19 ± 0.01 µm/hour, respectively (n=3 experiments). These observations suggest HeLa cell populations comprised of K+ cells have greater tumorigenic potential than those containing K- cells. The results provide insight about the influence of cytokeratin filaments on cell physiology beyond simply being a diagnostic measure of cervical cancer. Supported by the McNair Scholars Program (SP) and the COLSA Karabelas Fund (DHT).

Methods and Materials

Cell Proliferation Assay
- The two different strains of HeLa cells (i.e., K+ and K-) were initially grown to 70 percent confluency in flat-bottom, cell growth tubes (initial seeding concentration = 125,000 cells/tube)
- Growth monitored at 24 hour intervals for a total of seven days
- Cells counted using a hemocytometer and light microscope
- The experiment was independently replicated a minimum of 3 times (n≥3 experiments)
- The number of generations, multiplication rate, and generation time of the two phenotypes was calculated

Cell Invasion Assay
- Cells grown in 10% serum to 80% confluency
- Cells serum-starved for 24 hours and then seeded into transwell inserts
- After 6 hours of incubation in the absence/presence of a chemotactic agent (serum), cells migrating to underside of insert fixed, stained with Crystal Violet, and counted
- Inserts subsequently de-stained in 250µl of 70% ethanol, shaken for 10 minutes, and air-dried

Cell Migration Assay
- An average of ~240 hours were required for wounds in the K+ cultures to attain closure compared to ~144 hours for the K- cultures (Figure 2)
- Migration rate (Mean ± SEM) of K+ vs. K- cells was 4.88 ± 0.01 vs 4.19 ± 0.01 µm/hour, respectively (n=3 experiments).

Results

Cell Proliferation Assay
- Total number of generations for K+ vs. K- cells was 2.87 ± 0.09 vs. 1.61 ± 0.12 generations
- Multiplication rate for K+ vs. K- cells was 0.67 ± 0.06 vs. 0.40 ± 0.03 doublings per day
- Generation time for K+ vs. K- cells was 1.52 ± 0.15 vs. 2.52 ± 0.18 days (Figure 1).

Figure 1. Growth curves of K+ vs. K- HeLa cells in vitro. Average cell counts for K+ and K- cells grown over a 7 day period of culture depicted. The data reflect the averages (±SEM) for three independent experiments conducted in duplicate.

Cell Migration Assay
- An average of ~240 hours were required for wounds in the K+ cultures to attain closure compared to ~144 hours for the K- cultures (Figure 2)
- Migration rate (Mean ± SEM) of K+ vs. K- cells was 4.88 ± 0.01 vs 4.19 ± 0.01 µm/hour, respectively (n=3 experiments).

Figure 2. Wound healing assays to determine differences in cell migration between the K+ and K- HeLa cells. The area of the initial wound is illustrated by the solid black line. Closure of the wound area was quantified using QCapture software. A representative assay for one of the three independent experiments is depicted.

Results (Continued)

Migration area of K+ vs. K- HeLa Cells
- A preliminary experiment indicated K+ cells were more invasive in response to a chemotactic agent compared to K- cells (Figure 4).

Figure 3. Area of migration in wound healing assay for K+ vs. K- HeLa cells. Average migration area (±SEM) over the 6 day period of culture is depicted. Three independent experiments were conducted in duplicate. K+ cells reduced the “wound” and migrated over the surface area in a shorter amount of time than K- cells.

Cell Invasion Assay
- A preliminary experiment indicated K+ cells were more invasive in response to a chemotactic agent compared to K- cells (Figure 4).

Figure 4: Assessment of K+ and K- HeLa cell migration in a Transwell migration system (20X magnification). Cells migrating to the underside of the insert were counted manually. Greater numbers of migrated cells were observed for K+ cultures compared to K- cultures (*P<4 vs. 674 pm, respectively).

Figure 5: Measurement of K+ and K- HeLa cell migration as a function of crystal violet absorbance. Transwell inserts were de-stained in ethanol and absorbance read at 540nm.

Conclusions

Cytokeratin 8/18 filaments contribute to the aggressive growth and migration of HeLa cells, suggesting enhancement of tumorigenic potential
- Targeting the disruption or loss of cytokeratin 8/18 filament expression in cells may provide a therapeutic strategy to curtail further metastasis of cervical cancer

Figure 6: Intermediate Filament Structure

Contact
Stephanie Parisi (sta27@wildcats.unh.edu)
David H. Townson (dave.townson@unh.edu)

Acknowledgments
- Thank you to Dr. Townson and Foxall, and their laboratories, for their support and assistance with these studies
- The two phenotypes of HeLa cells used in this work were originally derived in the Townson laboratory
- This research was supported by the McNair Scholars Program (SP) and the COLSA Karabelas Fund (DHT)

References
1. Liao S, Lee WS, Chen H, Chuang L, Pan M, Chen C. Baseline human papillomavirus infection, high vaginal parity, and their interaction on cervical cancer risks after a follow-up of more than 10 years. Cancer Causes & Control 2012(5):703