Tracking plasticity-dependent cell cycle effects

Christian Y. Beke Onana[1], Luigi Perelli[2] & Giannicola Genovese[2,3]

[1] Partnership for Careers in Cancer Science and Medicine, Department of Faculty Diversity, Equity & Inclusion, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030.

[2] Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030.

[3] Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Introduction

- Cell plasticity is the ability of cells to change their phenotypes in response to intrinsic and extrinsic cues.
- Epithelial to mesenchymal transition (EMT) is a form of cell plasticity, well described in the context of human cancers, in which epithelial cells gradually lose canonical markers and acquire phenotypes similar to mesenchymal cells.
- The cell cycle is a series of growth and development steps a cell undergoes. It consists of two main phases: Interphase and Mitosis. The Interphase is further divided into the G_1, S, and G_2 phase.
- The effects of cell plasticity on the cell cycle program are poorly understood in the context of cancer.

Hypothesis: A cell having to deal with chromosomal abnormalities such as aneuploidy would need a longer G_2/M phase. We expect mesenchymal cells to have a longer G_2/M phase compared to epithelial cancer cells.

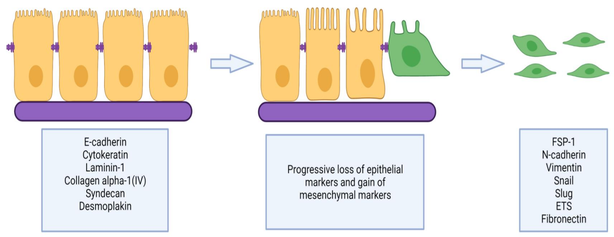

Figure 1. Epithelial-mesenchymal transition. Some epithelial and mesenchymal markers are listed (Adapted from Kalluri, R. & Weinberg, R.A., 2009). Created with BioRender.com.

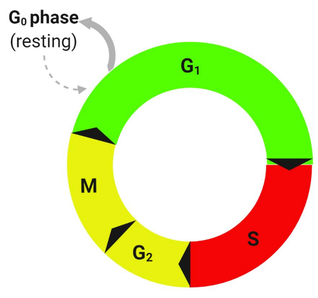

Figure 2. The various phases of the cell cycle; G_1, S, G_2 and M phases. Created with BioRender.com.

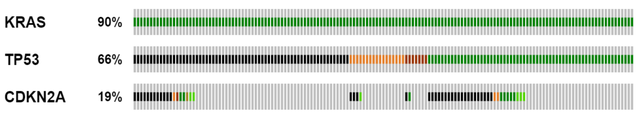

Figure 3. Mutation profile of pancreatic cancer (Bailey et al., 2016).

Materials/Methods

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- Pancreatic cancer cell lines were derived from genetically engineered mouse model (GEMM) $Pdx1^{Cre}$, $H11^{LSL\text{-}Cas9}$, $KRas^{LSL\text{-}G12D}$ after orthotopical injection of adeno-associated viral particles carrying guide RNAs for the knockout of Trp53, Cdkn2a, and Cdkn2b.
- To explore the various phases of the cell cycle and ensure a clear transition between the phases, we relied on a technique called Fluorescent Ubiquitination-based Cell Cycle Indicator using a pLenti-PGK-Neo-PIP-FUCCI (FUCCI) plasmid.
- Amplification of bacterial DNA via overnight growth in LB Broth medium and DNA extraction with QIAGEN kit (Cat. No 12162) according to manufacturer's protocol

Transfection of HEK293 and generation of Lentiviral particles

- Cells were plated with a 70% confluency in 150 mm petri dish. A mixture of OPTIMEM medium (1.7 ml), FUCCI (30 µg), psPAX2 (19.9 µg), PMD2G (9 µg), and PEI (110 µl) was added to the cell culture dropwise. The medium was changed 16 hours later.
- Two days later, the medium was collected, and lentiviral particles were isolated following two-step centrifugation protocol (first at 3000 RPM for 10 mins then 25000 RPM for 3 hours). The lentiviral particles were resuspended in 100 µl of PBS.

Infection of epithelial and mesenchymal cell lines

- GEMM-derived epithelial (EPI) and mesenchymal(MS-L) cell lines were plated at 50% confluency. The previously generated lentiviral particles were added dropwise to the GEMM-derived cells. The medium was changed following a 24 hour-period.

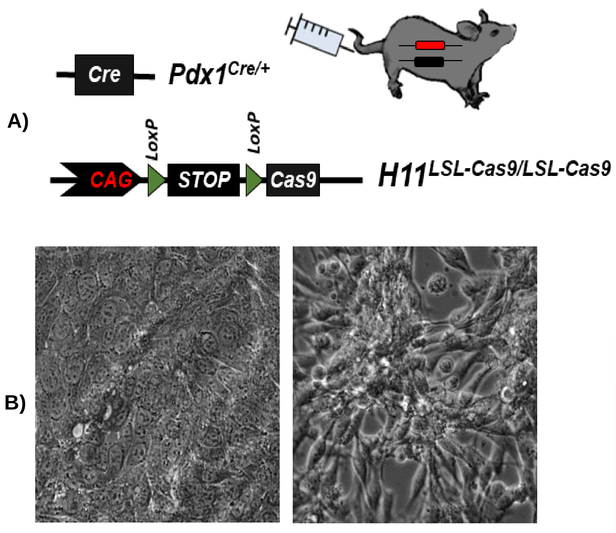


Figure 4.A) Generation of a genetically engineered pancreatic cancer model using the CRISPR/Cas9 technology to create knockouts of Trp53, Cdkn2a, and Cdkn2b and a KRas G12D mutation at the H11 locus. B) Epithelial (left) and mesenchymal (right) cell lines obtained from the genetically engineered pancreatic cancer model above viewed under Brightfield microscopy.

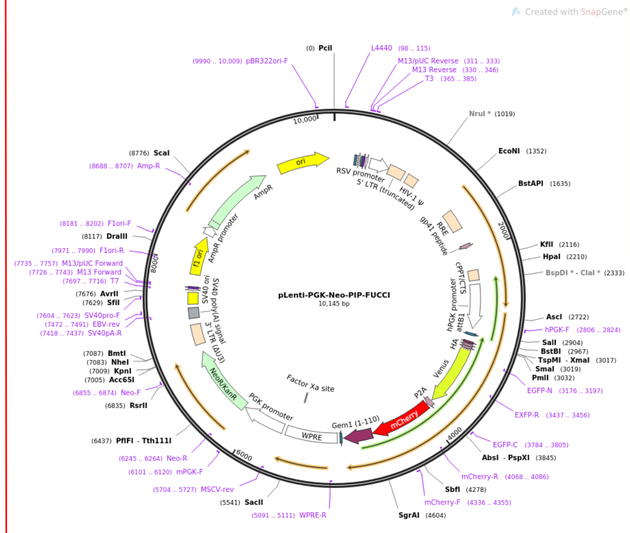
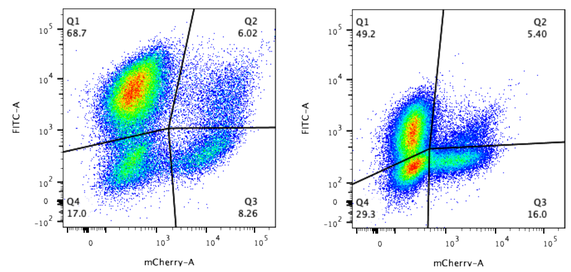


Figure 5. Full sequence map for pLenti-PGK-Neo-PIP-FUCCI plasmid (Addgene plasmid #118616, http://n2t.net/addgene:118616 ; RRID:Addgene_118616).

Results

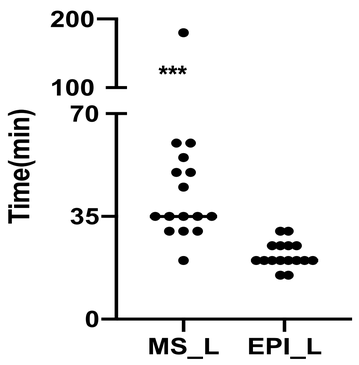
- GEMM-derived EPI and MS-L cell lines were efficiently transduced with FUCCI vector.
- Analysis of the cell cycle of these cell lines showed a prolonged G_2/M phase in the MS-L cells compared to the EPI cells.
- MS-L cells also exhibited catastrophic mitotic events that lead to aneuploidy.

A) FUCCI – distribution cell cycle

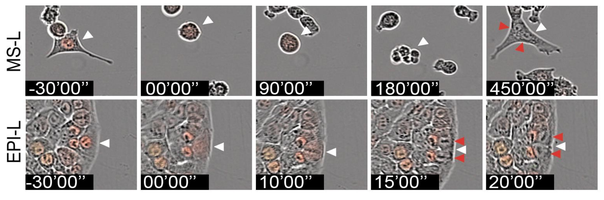


EPI-L MS-L

B) Time of Mitosis



C) Time of Mitosis



D) DNA CONTENT ANALYSIS

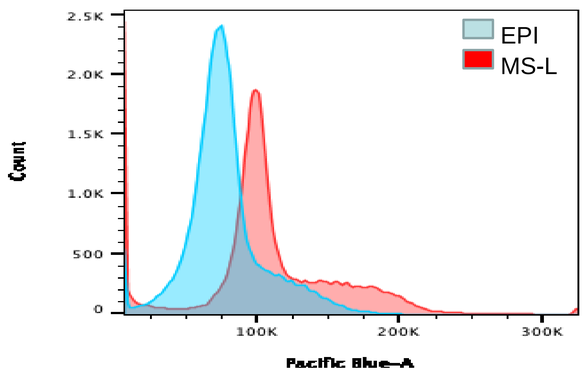


Figure 6. A) Scatterplot comparing our two fluorescent dyes, m-Cherry and FITC-A showing our various populations that are in various cell cycle phases. B) Statistically significant difference between EPI and MS-L cells in the length of mitosis C) Illustration of mitotic divisions along with fluorescence and catastrophic mitotic events observed in MS-L cells. D) DNA content in MS-L cells is more than the DNA content in EPI

Discussion

- Following Fluorescence-Activated Cell Sorting (FACS), we were able to isolate cells based on their fluorescence. We noticed that MS-L cells proliferate more as evidenced by a significant increase of cells in S-phase (16% vs 8.26%).
- The length of the G_2/M phase is longer in MS-L cells (35') compared to EPI-L cells (20') suggesting that these cells possibly have a higher DNA content and as such require more time to divide in order to handle that huge DNA content. There is also evidence of catastrophic mitotic events as evidenced by Figure 5C where we notice a cell with two nuclei.
- DNA content analysis confirms our hypothesis that the MS-L cells have a much higher DNA content compared to EPI cells probably due to an increased number of catastrophic mitosis.

Conclusions

- Pancreatic cancer is a deadly disease. Aneuploidy has been described as a common event in cancer priming tumor cells to metastasis (Bakhoum et al., 2018). In this study, we demonstrated an evident correlation between epithelial-mesenchymal transition and aneuploidy.

Future work

- Given this data, MS-L cells rely on specific modifications of cell cycle kinetics. Our goal, therefore, is to utilize these newly discovered vulnerabilities to find new therapeutic regimens that can reduce cancer burden.

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