Cell on the Run! Boolean Modeling of the Migrating Epithelial Cell and Induction of Mesenchymal-Epithelial Transition by OVOL2 and GRHL2

Ruth J. Ressler & Erzsébet Ravasz Regan

Biochemistry & Molecular Biology Program; Departments of Biology and Chemistry; The College of Wooster, Wooster, OH

THE COLLEGE OF WOOSTER

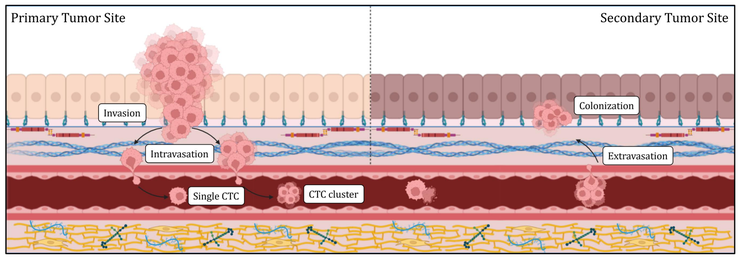
Ravasz-Regan Lab

ABSTRACT

Metastasis remains the main reason for therapy failure and contributes to 90% of cancer patient deaths. During this process, tumor cells respond to mechanical cues from the changing microenvironment and demonstrate phenotypic plasticity to survive the volatile journey to a distant, secondary tumor site. Epithelial-mesenchymal plasticity (EMP) has been used to explain how a tumor cell gains metastatic potential as well as tumor-initiating ability, also known as cancer cell stemness. However, the correlation between EMP, cancer cell stemness and the role of the tumor microenvironment remains elusive. To characterize the role of mechanosensitivity in EMP and cancer cell stemness, we included two phenotypic stability factors (OVOL2 and GRHL2) which have been linked to cell migration and stemness into our 155 node Boolean model. The construction of this model led to the finding that OVOL2 and GRHL2 stabilize a novel migrating epithelial phenotype in low to moderately dense cellular environments on stiff extracellular matrix. Our observations contrast previous reports on OVOL2 and GRHL2 expression stabilizing the hybrid epithelial-mesenchymal state, as we found these transcription factors to be dispensable in the maintenance of this phenotype. These findings are critical as they broaden the view on how a tumor cell demonstrates phenotypic plasticity in different microenvironments and can tune epithelial-mesenchymal markers to stabilize different states along the spectrum of EMP.

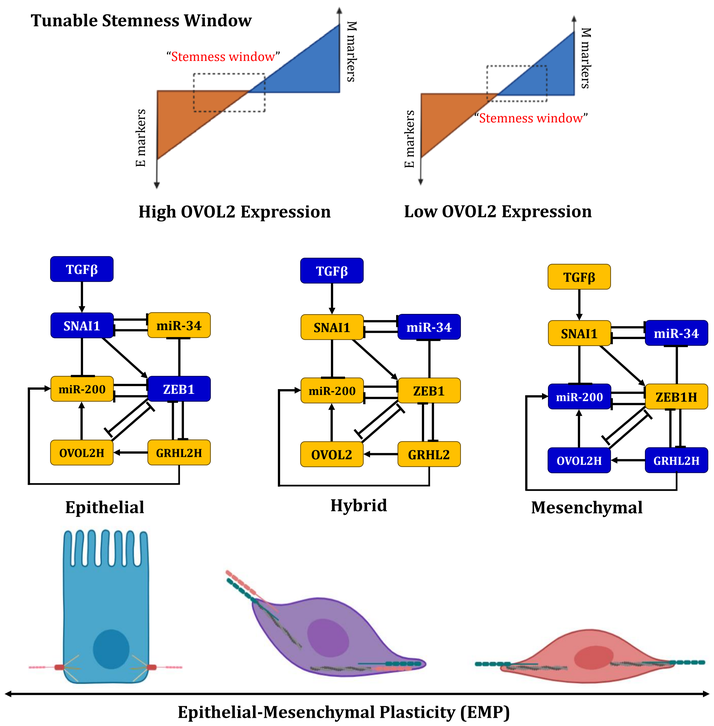
BACKGROUND AND SIGNIFICANCE

Depiction of Metastatic Cascade



Metastasis occurs when cancer cells from the tissue of the original tumor have migrated to a distant, secondary organ.

Signaling Molecules Tune Phenotypic Plasticity



Cancer cells undergo the reversible process of EMT to promote invasion, metastasis, and colonization. By tuning gene expression, cancer cells can gain the ability to migrate and form new tumors (stemness).

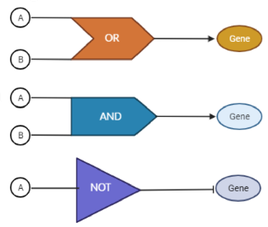
- Epithelial: apical-basal polarity; stable cellular junctions (E-cadherin)
- Mesenchymal: loss of cellular junctions; front-to-back polarity (N-cadherin)
- Hybrid: maintains some cellular junctions and can migrate

METHODS: BOOLEAN MODELING

Key Terminology
- Nodes: represent a molecule
- Edges/degrees: incoming or outgoing links

Assumptions
- Molecules are in high/low enough concentration to be active/inactive
  - Ignores rates and concentrations
- Attractors: stable state; phenotypes
- Synchronous update: nodes updated at **same time**

Logic Gates



FUTURE DIRECTIONS

- Building a stemness module by including lin28 and let-7 miRNAs
- Modeling intravasation and extravasation
- Simulating specific cancers by tuning gene expression to match published literature.

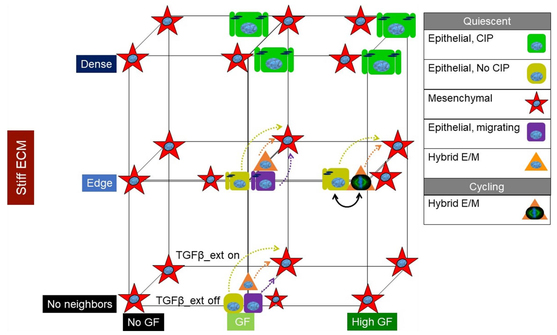
CONCLUSIONS

1. OVOL2 and GRHL2 may not be required to maintain the hybrid epithelial-mesenchymal phenotype but were found to stabilize a migrating epithelial phenotype in low cell density environments on stiff ECM.
2. High expression of OVOL2 or GRHL2 promoted the mesenchymal-epithelial transition, and in the case of high GRHL2, prompted partial MET.

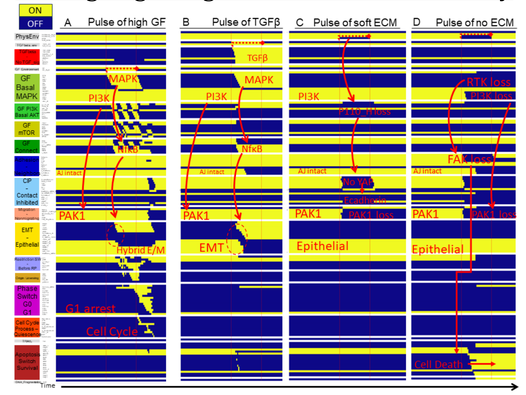
OVOL2 and GRHL2 may be phenotypic stability factors for the migrating epithelial phenotype due to their role in collective cell migration[1, 2].
Expression levels of OVOL2 and GRHL2 vary among cancer types, and within the same tumor [3], suggesting that cancer cells tune their gene expression to gain metastatic and/or tumorigenic potential dependent on mechanical and biochemical cues from the tumor microenvironment.

RESULTS

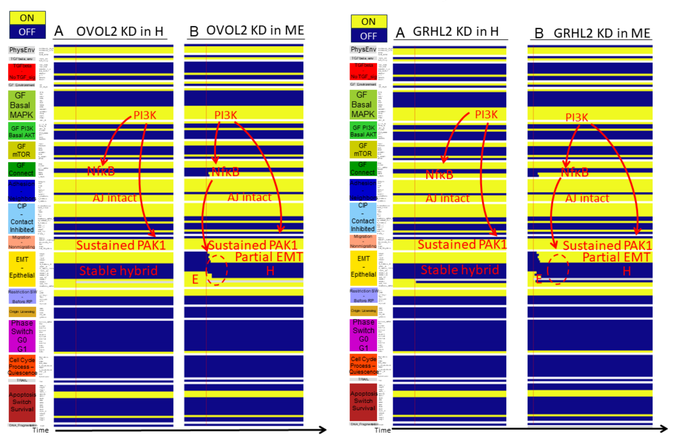
Attractor Map



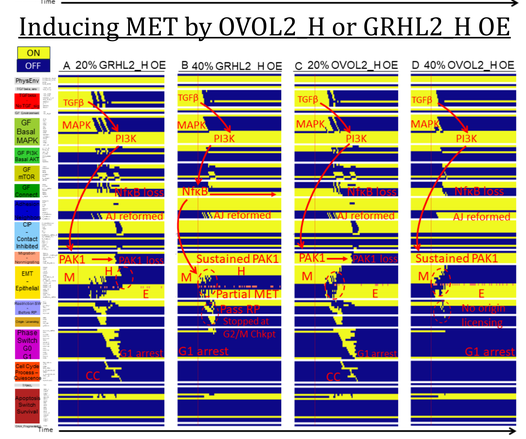
Testing Migrating E Cell Mechanosensitivity



OVOL2 and GRHL2 did not stabilize hybrid state



Inducing MET by OVOL2_H or GRHL2_H OE



References:
1. Varma, S., Cao, Y., Tagne, J.-B., Lakshminarayanan, M., Li, J., Friedman, T. B., Morell, R. J., Warburton, D., Kotton, D. N., and Ramirez, M. I. (2012) The Transcription Factors Grainyhead-like 2 and NK2-Homeobox 1 Form a Regulatory Loop That Coordinates Lung Epithelial Cell Morphogenesis and Differentiation *. J. Biol. Chem. 287, 37282–37295
2. Haensel, D., Sun, P., MacLean, A. L., Ma, X., Zhou, Y., Stemmler, M. P., Brabletz, S., Berx, G., Plikus, M. V., Nie, Q., Brabletz, T., and Dai, X. (2019) An Ovol2-Zeb1 transcriptional circuit regulates epithelial directional migration and proliferation. EMBO Rep. 20, e46273
3. Fu, H., Qi, L., Chen, L., He, Y., Zhang, N., and Guo, H. (2016) Expression of Ovol2 is related to epithelial characteristics and shows a favorable clinical outcome in hepatocellular carcinoma. *OncoTargets Ther.* **9**, 5963–5973