In Vitro Characterization of Proteins Associated With Cell Migration and Invasiveness in a Murine Model of Cancerous Progression

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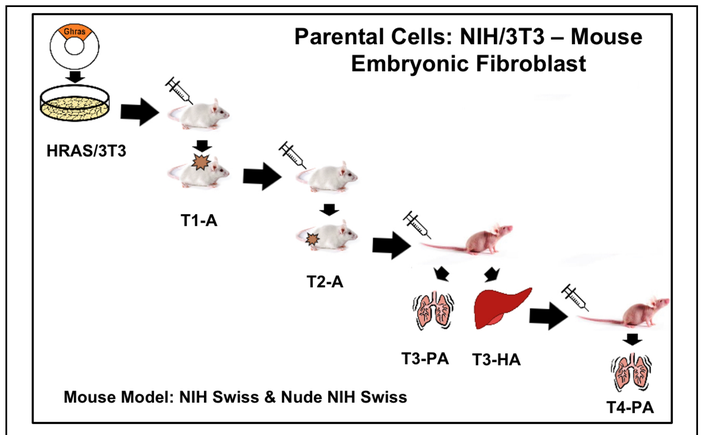
Introduction

Most cancer deaths are caused by advanced, metastatic stages of tumorigenesis. Therefore, there is a great need to have an ideal model system to study the complex development of tumorigenesis. The Ray/Jones Metastasis Model is comprised of seven cell lines developed to better research the transformation of cancer from a non-tumorigenic cellular phenotype to a highly aggressive metastatic phenotype. Each cell line represents a different stage of tumorigenesis, and the behavior of the cell lines matched the expected phenotype based on the stage of the tumor they were derived from. Further investigation of metastatic related proteins, such as Semaphorin-7A and Voltage Gated Sodium Channels are needed to confirm their role in cancer behavior.

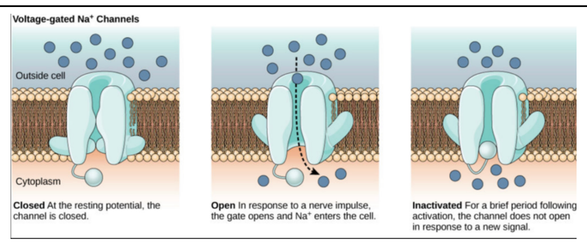
Model System



Drs. Durwood Ray and David Jones produced a novel six cell line model composed of progressively advanced cancerous lines all derived from a single non-tumorigenic cell line.

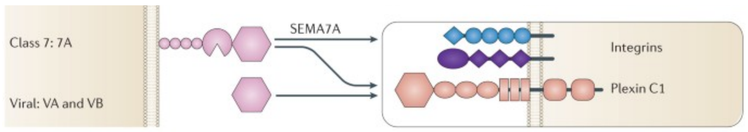
- **NIH/3T3:** parental murine embryonic fibroblast cell line.
- **HRAS/3T3:** NIH/3T3 were transferred to stably express a human HRAS oncogene.
- **T1-A:** derived from a primary tumor that formed HRAS/3T3 cells injected subcutaneously into an NIH Swiss mouse.
- **T2-A:** derived from a local metastasis on the rump of a NIH Swiss mouse injected in the tail vein with T1-A cells.
- **T3-PA:** derived from a distant lung metastasis from a nude NIH Swiss mouse injected in a tail vein with T2-A cells.
- **T3-HA:** derived from a distant liver metastasis from a nude NIH Swiss mouse injected in a tail vein with T2-A cells.
- **T4-PA:** derived from a distant lung metastasis from a nude NIH Swiss mouse injected in a tail vein with T3-HA cells. These produced numerous tumors in the organs and extremities when injected into subsequent NIH Swiss mice.

Voltage Gated Sodium Channels



- Voltage Gated Sodium Channels (VGSCs) are transmembrane proteins that allow the flow of sodium ions through a membrane.
- VGSCs have been well studied and characterized, but mostly in nerve and muscle cells, so more research is needed to confirm its role in the metastasis and invasiveness of cancer.
- Recent studies have shown that they are known to be upregulated in highly metastatic carcinomas.
- Based on this, the prediction is that there would be an upregulation of VGSCs expression in the cell lines.

Semaphorin-7A



- Membrane proteins that are identified as axonal growth cone guidance molecules.
- Signaling protein that is associated with driving the invasion and survival of postpartum breast cancer.
- Recent studies show it is associated with the metastasis and inflammation of primary tumors to different organs.
- Previous study shows that it's seen upregulated in five out of six lines and downregulated in one of the six lines relative to NIH/3T3 based on proteomics.

Experimental Design

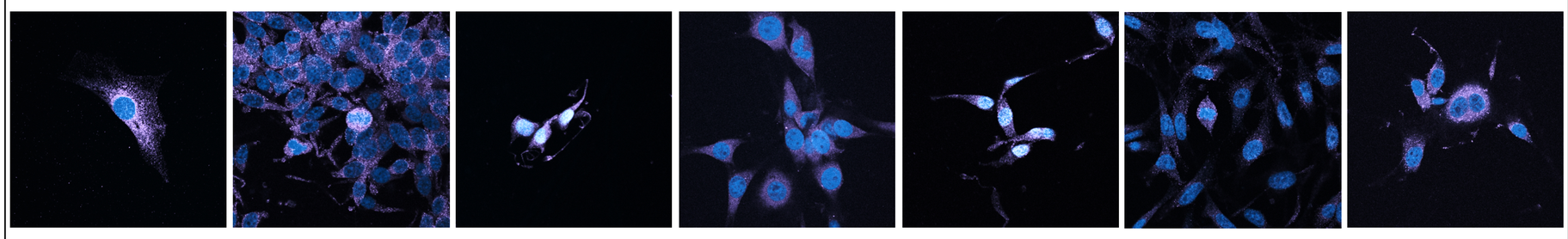
Staining:

- Primary Antibody: 1:200
  - Anti-Pan Na_V
  - Anti-Semaphorin-7A
- Secondary Antibody: 1:1200
  - Alexa 488
- Counter-Nuclear Stain: 1:600
  - DRAQ5

Results

Voltage Gated Sodium Channels

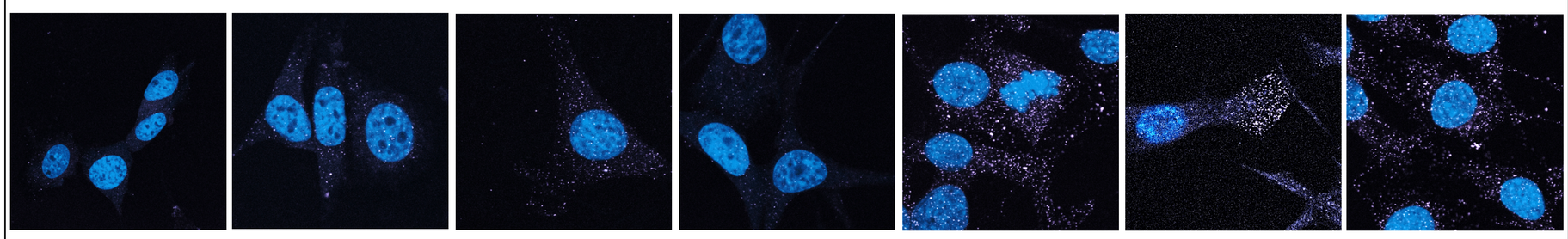
40x objective oil immersion*



NIH/3T3 | HRAS/3T3 | T1-A | T2-A | T3-HA | T3-PA | T4-PA

Semaphorin-7A

100x objective oil immersion*



NIH/3T3 | HRAS/3T3 | T1-A | T2-A | T3-HA | T3-PA | T4-PA

Semaphorin-7A Proteomics

		Abundance Ratio - Normalized to NIH/3T3					
Accession	Protein Name	HRAS/3T3	T1-A	T2-A	T3-HA	T3-PA	T4-PA
Q9QUR8	Semaphorin-7A	10.318	10.481	0.27	26.02	32.082	20.621
Function	Mediates integrin-mediated signaling; regulates Cell migration and immune responses.						
Description	Drives invasion and survival of postpartum breast cancer.[41]						

Conclusion

- There is expression of voltage gated sodium channels (VGSCs) in all the cell lines, but it cannot yet be said that there is an increase in VGSCs expression as the cell lines become more metastatic.
- There is expression of Semaphorin-7A in all the cell lines, correlating with the proteomic data gathered from a previous study done by the lab.

Future Directions

- Western Blot: quantitatively measuring how much VGSCs and Semaphorin-7A are expressed in each cell line.
- siRNA Knockdown: blocking Semaphorin-7A and doing a motility/invasiveness assay to see how the lack of Semaphorin-7A affects cancer's behavior.
- Focusing more on VGSCs isoforms such as Na_V 1.4, 1.5, etc in order to narrow down focus points and targets.

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