Differential Effects of Oncogenic Ras Isoforms on Epithelia

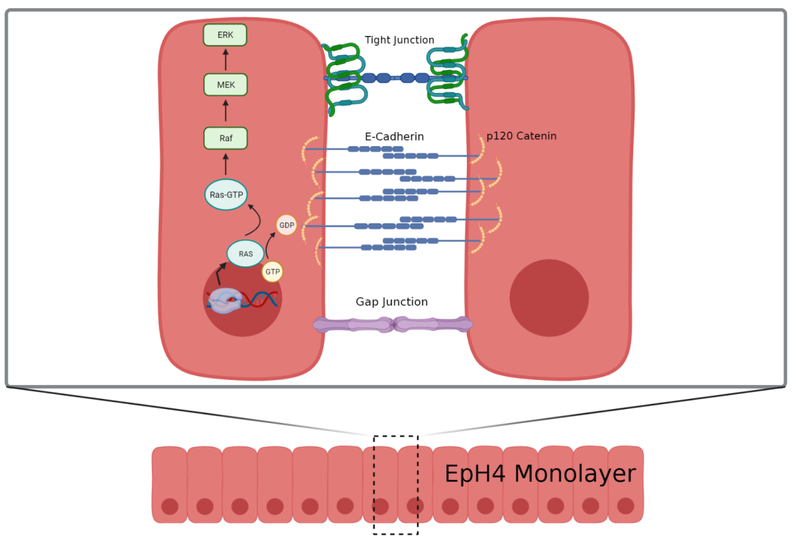
1.

About the Project

For my Regeneron STS project, I used mouse derived mammary gland cells to model the earliest stages of tumorigenesis. While tumors and cancers are generally thought of as long term processes, I uncovered, characterized, and worked to define some of the earliest changes in Ras mutated cells. Ras is a small signaling protein that is linked with many growth and cell cycle signaling pathways. It is no wonder that 19% of all cancers will harbor a Ras mutation, making this a protein of great importance and interest. I found that in less than 24 hours after inducing a mutated Ras, cells began to remove a protein called E-Cadherin from their membranes. Not only was this phenomenon rapid in nature but also fits into the context of abnormal cellular behavior. Since E-Cadherin normally acts as a "glue" between neighboring cells, this finding could explain why certain cancerous cells are more likely to detach from neighboring cells and float off. Cells that are less tightly connected to neighboring cells could be especially prone to metastasize. In addition, I also worked to uncover the specific (as there are many Ras associated pathways) Ras pathway that was linked with E-Cadherin internalization and thus showed that it was possible to stop cells from removing E-Cadherin from the membrane even after expressing mutated Ras. In the final parts of my research, I identified a key protein that explains an early mechanism behind E-Cadherin internalization: p120 Catenin. I am currently working on better understanding how activated ERK, p120 catenin, and E-Cadherin all tie together to formulate a mechanism behind E-Cadherin internalization in early Ras transformed epithelia.

2.

Background



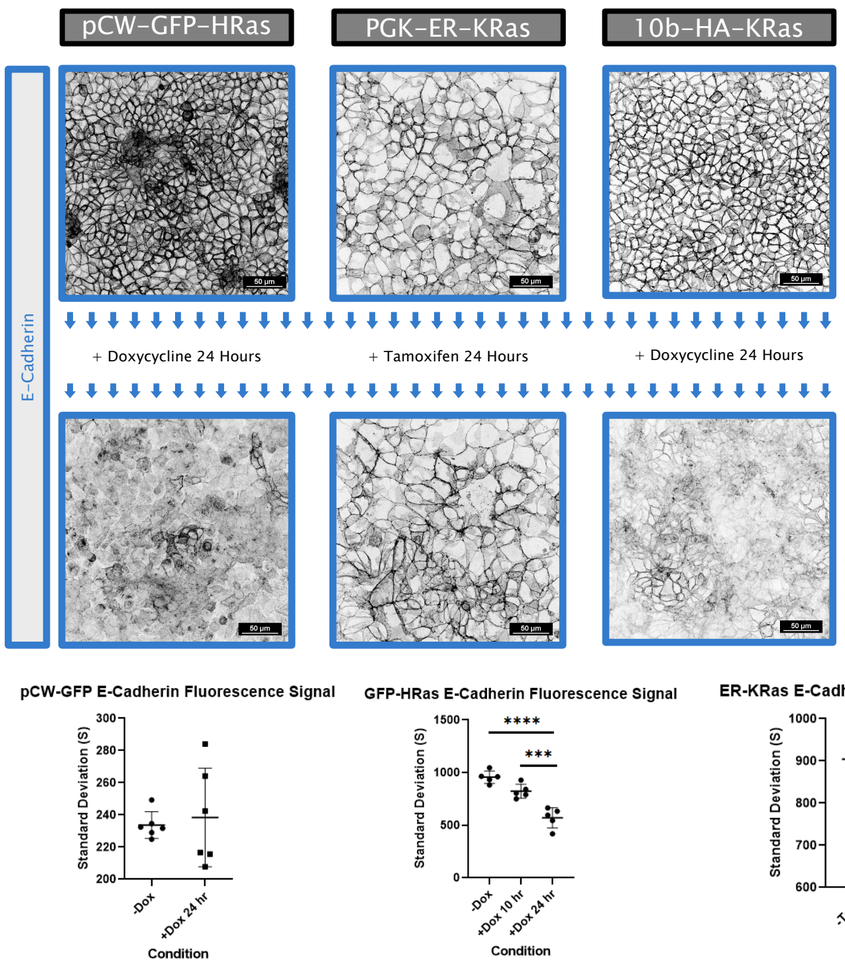
Epithelia are a specialized tissue that line almost all internal and external surfaces in the body. Epithelia have among the highest turnover rate compared to almost all other tissues while also needing to maintain structural morphology and protein polarity. Shockingly, as many as 90% of all cancers arise from epithelial cells and are labeled carcinomas.

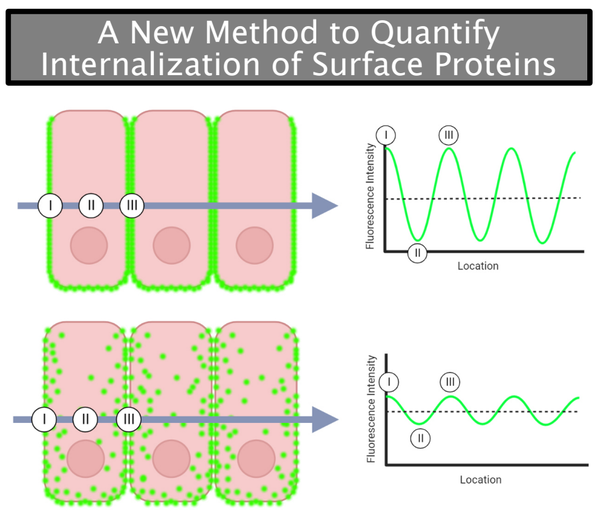
Ras GTPases are signaling proteins present in all animal cells and play a crucial role in many proliferation and growth pathways; however, when mutant Ras is constitutively active, it can lead to hyperplasia and tumorigenesis.

E-Cadherin belongs to a groups of Cadherin proteins that form interactions with other Cadherin proteins from neighboring cells by pairing up as seen in the model. E-Cadherin plays an essential function in epithelial cell polarity, tissue integrity, and the maintenance of a monolayer. It also functions as a tumor suppressor through contact inhibition, which allows cells to sense when there are neighboring cells, and it is frequently lost in invasive carcinomas.

p120 Catenin is a catenin protein that when it is bound to the cytoplasmic tail of E-Cadherin, works to inhibit the endocytosis of E-Cadherin, hence stabilizing it at the membrane. Because of this, p120 is known to be important in the maintenance of cell-to-cell adhesion.

3.





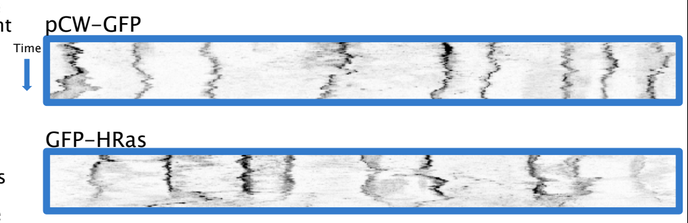
Since the total amount of E-Cadherin should remain unchanged, the mean fluorescence intensity μ across a line should also be the same in induced versus uninduced cells. However, the standard deviation of the fluorescence intensity changed based on the location of signal across the line. More steady signal leads to lower peaks and higher troughs, thus reducing σ.

4.

Immunofluorescent Staining is relatively quick and simple; however, there are also trade offs associated with this convenience. The most important one being that fixed cells ex. using paraformaldehyde kills the cell, meaning that important biological processes or functions of living cells that could be important in E-Cadherin localization are no longer present; some potentially important contextual information is gone. Secondly, fixation captures a moment in time, but protein interactions and behavior are highly dynamic.

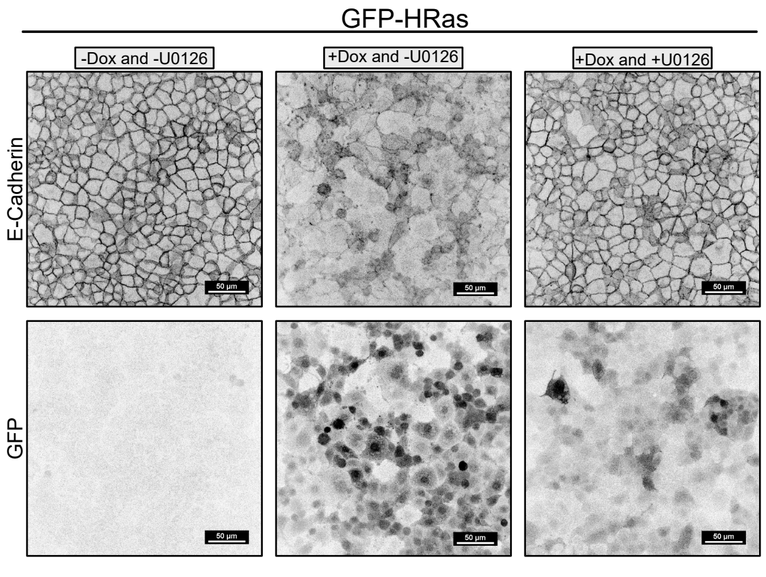
- Over expressed E-Cadherin-TD Tomato
- Knock down Endogenous E-Cadherin

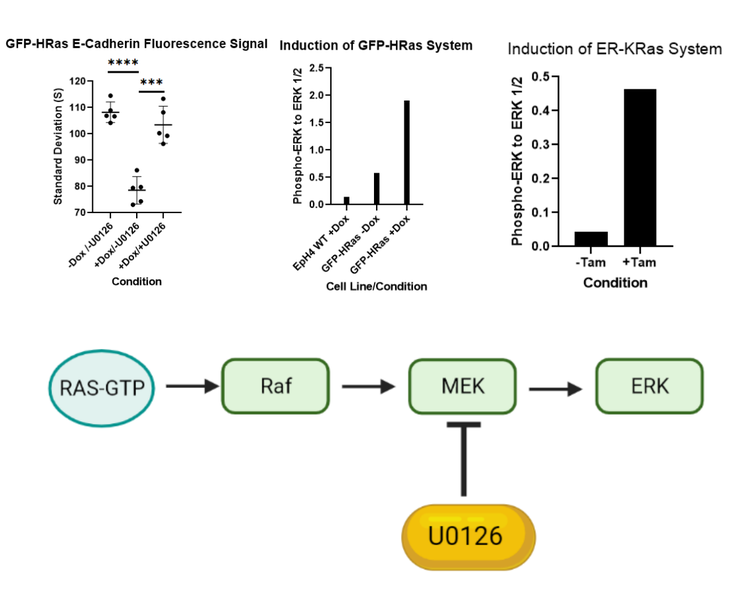
Kymographs show position across a period of time. The X axis can be interpreted as the position of E-Cadherin across a line, and the Y axis can be interpreted as time. The top of the y axis marks the beginning of the recording period, and moving down the y axis reflects an increase in time. In these kymographs, E-Cadherin localization across multiple cells are tracked in Ras vs control cells. While there is never a disappearance of membrane associated E-Cadherin in control cells, there is a sudden disappearance of E-Cadherin in Ras cells showing that once this internalization process begins, it depletes the cell of surface E-Cadherin quickly.



5.

The MAP Kinase (MAPK) pathway is a major signaling pathway resulting from Ras activation. While often linked to proliferation, it turns out that it is also the key pathway necessary for E-Cadherin internalization. Other pathways tested included the Rho-Kinase pathway, related to cytoskeleton contractility, and the AKT pathway related to cell growth, survival, and other functions. These pathways, even when inhibited, proved to have no effect on E-Cadherin distribution during Ras transformation.





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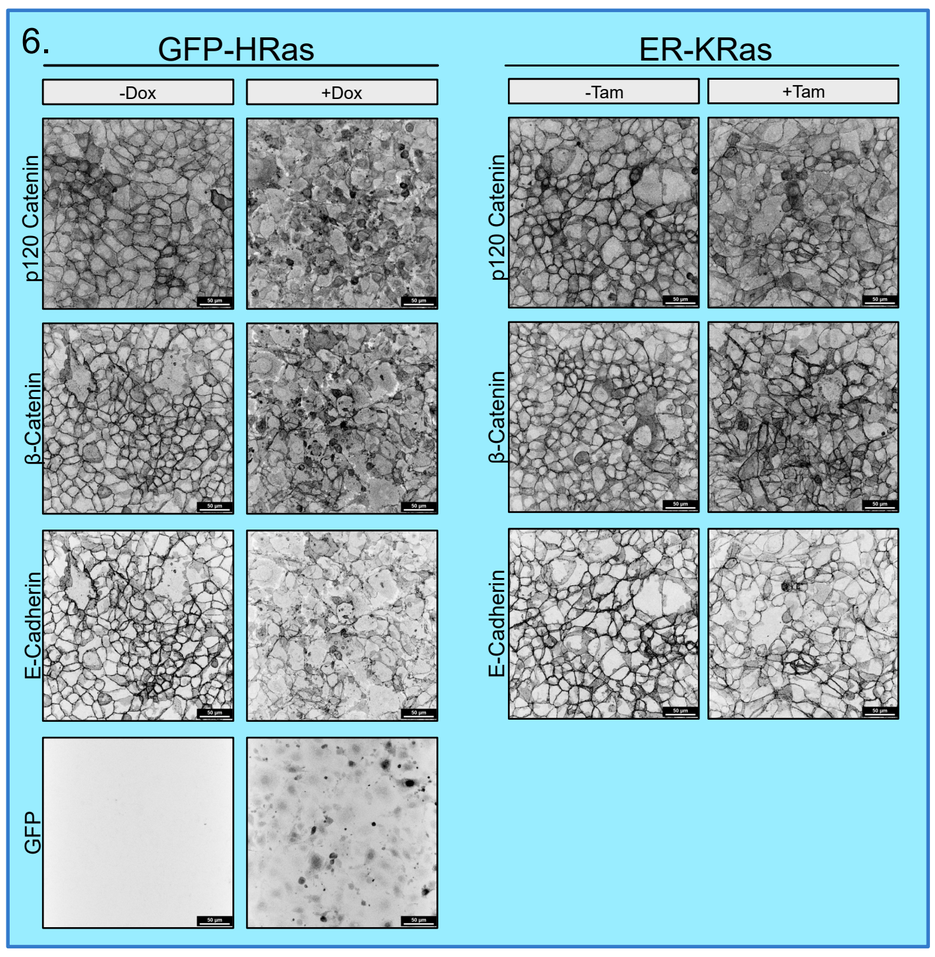

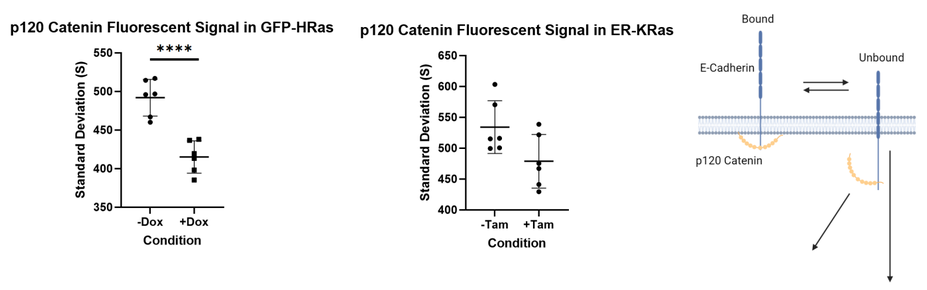

7.

Current Progress

I am actively working with the Mass Spectrometry Core Lab to conduct a phosphoproteomics study and search for ERK phosphorylation sites on p120. I hypothesize that ERK driven phosphorylation of p120 could cause it to "fall off" of E-Cadherin, allowing it to be internalized.

I am using a "phospho-dead" p120 mutant where 6 known p120 phosphorylation sites have been target with a single amino acid point mutation to Alanines. Subsequently, I will use CRISPR/Cas9 to Knock out endogenous p120 to probe for whether or not p120 phosphorylation is a key step in E-Cadherin internalization.

8.

Conclusion

I have shown that H-Ras and K-Ras4b, two Ras isoforms, signal through the same pathways yet can lead to a variety of phenotypes seemingly correlated with activate ERK levels. Furthermore, I identified that the MAPK pathway is essential to the internalization of E-Cadherin but that additional components are required in order to achieve the E-Cadherin internalized phenotype. By taking a structural view of the E-Cadherin intracellular complex, I was able to correctly identify and observe the simultaneous internalization of both p120 Catenin and Beta-Catenin. This leads to the idea that there must be effects of Ras driven transformation that extend beyond the direct and limited effects on E-Cadherin.

My research has shown that while the role of Ras in the context of transformational changes has been assumed to be a long term process, it can actually occur in a very short period. The appreciation for early timeline events is not something greatly reflected in molecular biology, especially when it comes to tumorigenesis and carcinogenesis. Although such processes do occur over time, understanding their initial behaviors could prove to be more effective in stopping or slowing their spread compared to a later time period when such invasive cells have already begun to spread and proliferate beyond control.

References

All cartoon images were created with BioRender, and all other images were independently taken
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