**Lung Adenocarcinoma (LADC)** is a cancer that initiates in epithelial cells within the outer part of the lung. This disease is classified as a Non-Small Cell Lung Cancer, which is the most common type of all lung cancers1. Ultimately, tumor growth spreads during metastasis causing fatigue, lack of appetite, coughing, reoccurring respiratory infection and sometimes death2. Currently, treatments include Tyrosine Kinase Inhibitors that target overactivated proteins like the Epidermal Growth Factor Receptor (EGFR), which are usually overactivated in cancerous tissues. Specifically, mutations in EGFR’s tyrosine kinase domain can result in constitutive autophosphorylation, which can affect epithelial cell cycle and transcription of cell’s adhesion, polarity and ability to differentiate 4. These changes in gene expression and function contribute to epithelial cell transitions, which may affect sensitivity to TKI treatment3. *It’s still unclear how EGFR tyrosine kinase mutations influence cell adhesion and polarity.*

**My objective** is to determine whether EGFR tyrosine kinase mutations contribute to different levels of invasion, differentiation, polarity and adhesion. The **primary goal** of this study is to find whether EGFR mutations change expression of genes related to acquired resistance towards inhibitors. I **hypothesize** that tyrosine kinase mutations will ultimately result in tumor growth accompanied by increased transcription overexpression of invasion associated proteins.

**Aim 1: Verify the tyrosine kinase domain’s importance in overall cancer progression.**

**Rationale:** Ensemble and ClustalOmega multiple sequence alignment yield known homologs of the EGFR gene, including common model organisms such as drosophila. The resultant conserved proteins occurred in mammalian organisms. Furthermore, EGFR’s function in the nucleus is significantly more complex in mammals, making mice a better model organism5.

**Approach:** CRISPR Activation Plasmids will induce constitutive phosphorylation of EGFR in mice embroyos6. Verification of activation promoter insertion will be verified by DNA sequencing of transfected mouse tissue to confirm the incorporation of the plasmid and absence in wildtype. Mouse respiratory health will determine whether the mutation has affected vital organs7.

**Hypothesis:** I hypothesize that tyrosine kinase domain overactivation will result in weight loss, circulating tumor cells and reoccurring respiratory infection.

**Aim 2: Identify how EGFR overactivation affects gene expression associated with epithelial transition.**

**Rationale:** Changes in invasion markers, like E-adherin and Vimentin between wildtype and transgenic mice indicate cell transition between invasive mesenchymal cells3. Changes in transcription of cell these adhesion molecules can determine the extent of cell transformation upon EGFR mutation.

**Approach:** Performing a microarray using cDNA library with tumor cells extracted from mouse epithelial tissue, determining whether expression have changed upon mutation. Finally, Gene Ontology analysis using PANTHER can enrich gene expression with GO terms relating to epithelial adhesion.

**Hypothesis:** I hypothesize that EGFR mutations ultimately result in an increase of mRNA expression of known epithelial transition associated proteins.

**Aim 3: Determine how EGFR overactivation affects specific protein interaction.**

**Rationale:** Overactivation of EGFR are correlated with increased translation and complexation with mesenchymal markers indicative of epithelial transition. For example, overexpressed Mucin-1 associates with overactive EGFR8, which has been linked to TKI sensitivity9.

**Approach:** SILAC metabolic labeling will quantify specific binders to EGFR between wildtype and transfected mice. Samples will be subject to LC-MS/MS spectroscopy separating heavy and light peptides, thereby elucidating contaminants from specific binders to EGFR.

**Hypothesis:** I postulate that epithelial associated proteins that show increased gene expression will specifically bind to overactive EGFR.

**References:**

1. Cancer Treatment Centers of America, Lung cancer types, pg 2. Retrieved from: <https://www.cancercenter.com/cancer-types/lung-cancer/types>
2. Zhang, Natasha B. Leighl, et. al (2019). Emerging therapies for non-small cell lung cancer [Journal of Hematology and Oncology Article], pg 1. Retrieved from: <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-019-0731-8>
3. Uramoto, Iwata, et. al (2010). Epithelial–Mesenchymal Transition in EGFR-TKI Acquired Resistant Lung Adenocarcinoma [Pubmed Article]. Retrieved from: https://www.ncbi.nlm.nih.gov/pubmed/20682976
4. Sven Bogdan, Klämbt, Epidermal growth factor receptor signaling (2001), pg 3. Retrieved from: [https://www.cell.com/current-biology/comments/S0960-9822(01)00167-1](https://www.cell.com/current-biology/comments/S0960-9822%2801%2900167-1)
5. Tian, Shi, et. al (2018). Different subtypes of EGFR exon19 mutation can affect prognosis of patients with non-small cell lung adenocarcinoma [Pubmed Article]. Retrieved from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6211626/>
6. Ohashi, Rai, et al., Induction of lung adenocarcinoma in transgenic mice expressing activated EGFR driven by the SP‐C promoter (2008). Retrieved from: https://www.ncbi.nlm.nih.gov/pubmed/18564139
7. Sawabata, Circulating tumor cells in lung cancer: cluster circulating tumor cells as hybrid epithelial-mesenchymal transition/mesenchymal-epithelial transition (2017). Retrieved from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5723813/>
8. Lin, Wang, et al., EGFR-TKI resistance in NSCLC patients: mechanisms and strategies (2014). Retrieved from: <https://www.nature.com/articles/onc2012158>
9. Singh, Behrens, et al., Phosphorylation of MUC1 by Met Modulates Interaction with p53 and MMP1 Expression (2008). Retrieved from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4163608/>