Determining the contribution of cystathionine beta synthase to lung cancer lineage fate

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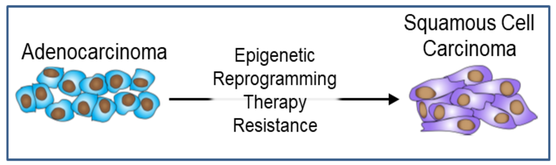
Hypothesis

We hypothesize that increased activity of the enzyme cystathionine beta synthase (CBS) can drive epigenetic reprogramming of lung adenocarcinoma (ADC) to an aggressive and therapy-resistant state through reduction of S-adenosyl methionine (SAM) pools and destabilization of Polycomb Repressive Complex 2 (PRC2), leading to expression of squamous genes. We are testing this hypothesis in human cells and genetically engineered mouse models.

A)

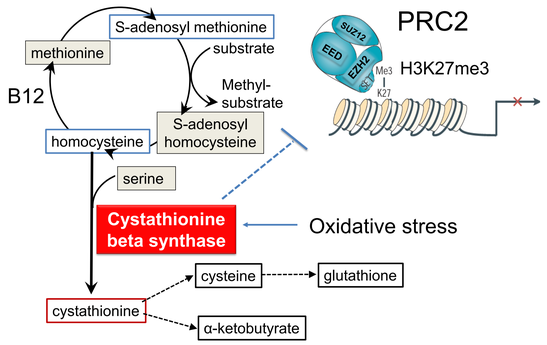
Background

Lung cancer is a devastating disease with high mortality even when diagnosed at an early stage. The two major pathologies of non-small cell lung cancer, adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are historically treated as separate diseases, even though mounting evidence shows that epigenetic reprogramming from ADC to a more SCC fate allows lung cancers to evade therapies.

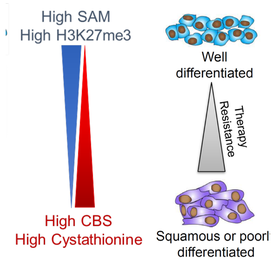


B)

Homocysteine Metabolism and Lung Cancer Lineage Fate



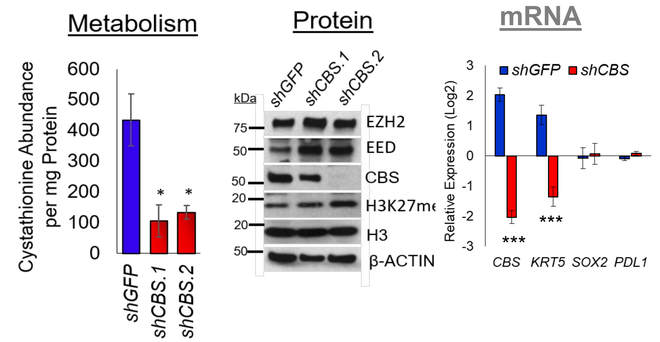
Our published work (Xu, Fillmore *et al.*, Cancer Cell 2014) demonstrated that lung SCCs have much higher cystathionine and lower S-adenosyl methionine (SAM) than ADCs. This observation lead to our hypothesis that activity of the enzyme CBS contributes to epigenetic reprogramming through destabilization of PRC2, which relies upon SAM levels for protein stability.



Levels of SAM influence PRC2 stability and activity, ADC has high SAM and SCC has high Cystathionine. increase chemotherapy response of lung cancer cells through the enzyme CBS and histone methylation changes .

C)

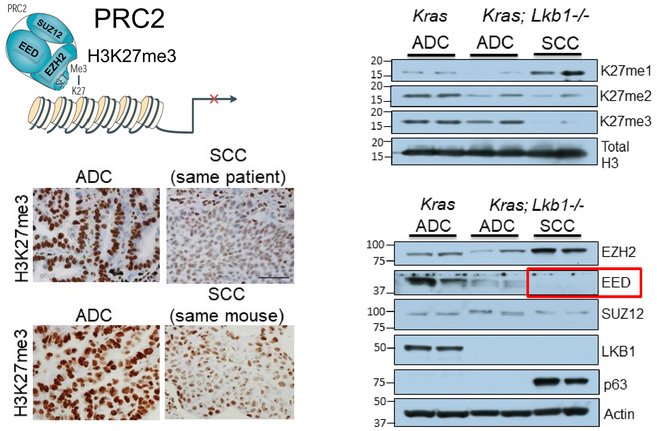
CBS Knock-Down Stabilizes the PRC2 Complex and Leads to Decreased Squamous Gene Expression



Here, we demonstrate that reduction of CBS by small hairpin encoding lentivirus leads to significantly decreased cystathionine levels, stabilization of EED and H3K27me3 and lower expression of the squamous genes *SOX2* and *KRT5*.

D)

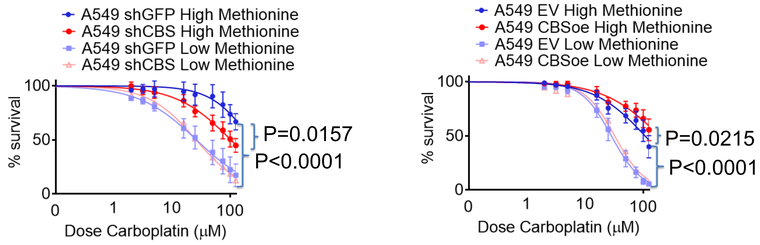
Polycomb Repressive Complex 2 Activity in ADC and SCC



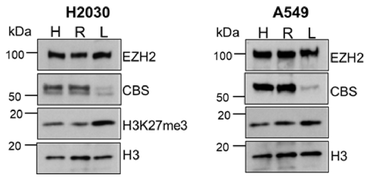
Our published work (Zhang, Fillmore Brainson *et al.*, Nat Comm 2017),(Bracken et al.,2009) demonstrated that squamous cell carcinomas have markedly reduced PRC2 function as measured by Histone H3 Lysine 27 tri-methylation, when compared to adenocarcinomas, even within the same patient. Loss of PRC2 leads to de-repression of squamous genes, including *p63*, *KRT5* and *SOX2*. The PRC2 subunit EED is the one that appears to be lost at the protein level in SCC.

F)

CBS Modulation, Coupled with Methionine Availability, Influences Carboplatin Sensitivity



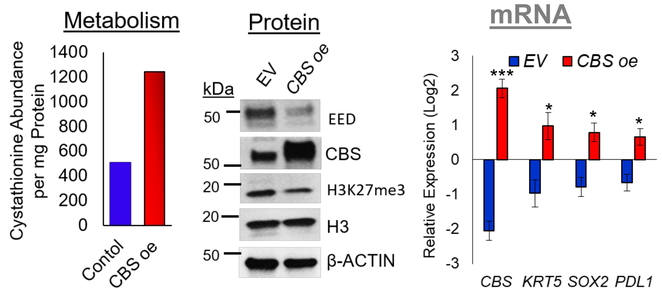
Here, we demonstrate that CBS expression levels influence response to the common chemotherapy carboplatin. Over-expression of CBS causes carboplatin resistance, while knock-down of CBS, or reduction of CBS through methionine restriction, sensitizes cells to carboplatin.



Regular Methionine: 17.24mg/L
Low Methionine : ½ R
High Methionine : 5 times R

G)

CBS Over-Expression De-stabilizes the PRC2 Complex and Leads to Squamous Gene Expression



Here, we demonstrate that over-expression of CBS by lentivirus leads to increased cystathionine, decreased EED protein, decreased H3K27me3 mark and increased expression of squamous-associated genes including *SOX2* and *PD-L1*.

Conclusions

Our data suggest that lung cancer lineage fate may be governed in part through expression and activity of the enzyme CBS. Because CBS is a redox-sensitive enzyme, this could be the missing link between increased oxidative stress and ultimate epigenetic reprogramming to a therapy-resistant squamous state in lung cancer. CBS modulation influences response to carboplatin, which could be leveraged therapeutically through methionine restriction. *In vivo* studies to test this hypothesis are underway.

Funding

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