Characterization of Intracellular and Extracellular Metabolites in Squamous-Cell Carcinoma (SCC)

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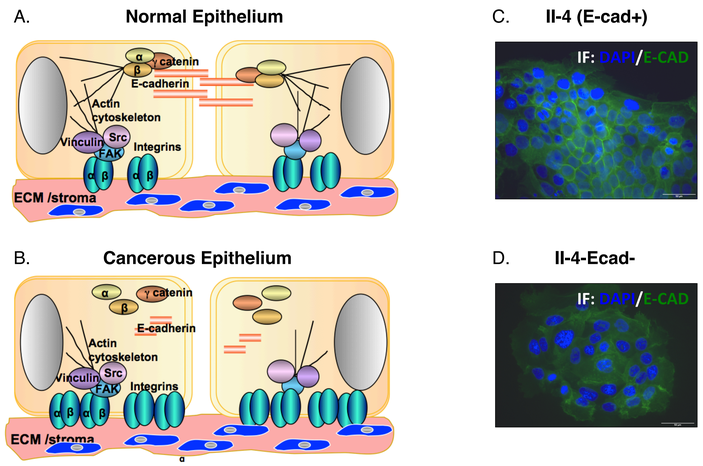
Introduction

Squamous cell carcinoma (SCC) is the cancer of the squamous cells found in the epithelium. Common prophylactic measures are proven to be ineffective in decreasing risk of SCC development. Nevertheless, it is known that loss of the cell-cell adhesion molecule and known tumor suppressor, E-cadherin (E-cad), is a hallmark of invasive SCC.

Background

Fig 1. E-cadherin-based adherens junction and E-cadherin loss in SCC[1]



Cell-cell adhesion as facilitated by **E-cadherin** in normal epithelium (A). Cancer cells lose cell-cell adhesion due to loss of E-cadherin (B). Immunofluorescence (IF) staining of **E-cadherin** and **cell nuclei**. Defined E-cadherin expression in cell-cell junctions leads to compact cell cluster (C). Loss of E-cadherin leads to cell dispersion (D).

Aim

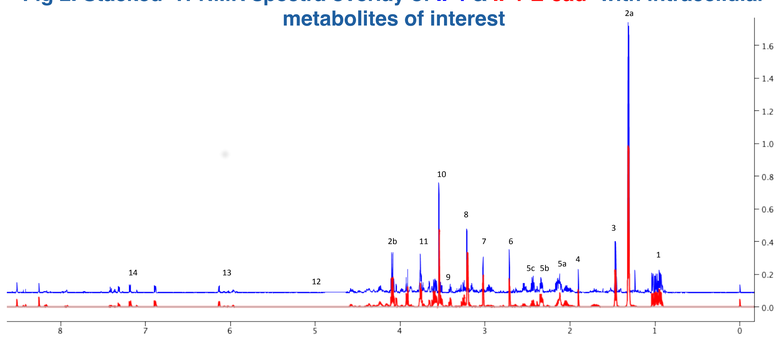
To determine if there are metabolic differences between Il-4-E-cad+ and Il-4-Ecad- SCC, and characterize the intracellular and extracellular metabolites via ^{1}H-NMR spectroscopy to establish a potential biomarker for invasive squamous cell carcinoma.

Methods and Materials

- Two cell lines were used: E-cadherin competent SCC (Il-4) and E-cadherin suppressed (Il-4-Ecad-) cells. Cell lines derived from *ras*-transformed human keratinocytes (HaCaT).
- Cells were grown in RPMI-1640 (HEPES modified) media for 48 hrs. 1.5 million Il-4 and Il-4-Ecad- cells were collected and plated in triplicate.
- Intracellular metabolites were collected through methanol extraction. Conditioned media was also collected.
- 600 MHz ^{1}H-NMR spectroscopy and CHENOMX were used to determine the concentrations and identity of intracellular and extracellular metabolites. Concentrations were normalized by sum to account for variable extraction efficiency. MetaboAnalyst was used for statistical analysis.

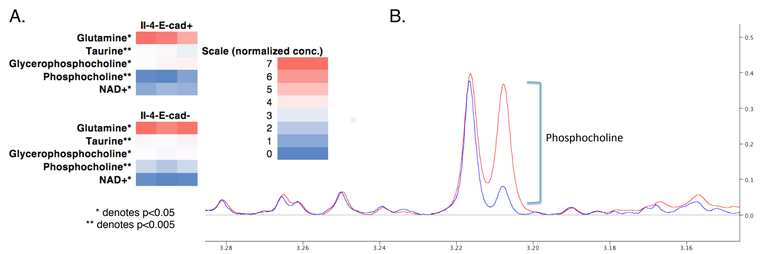
Results

Fig 2. Stacked ^{1}H-NMR spectra overlay of Il-4 & Il-4-E-cad- with intracellular metabolites of interest



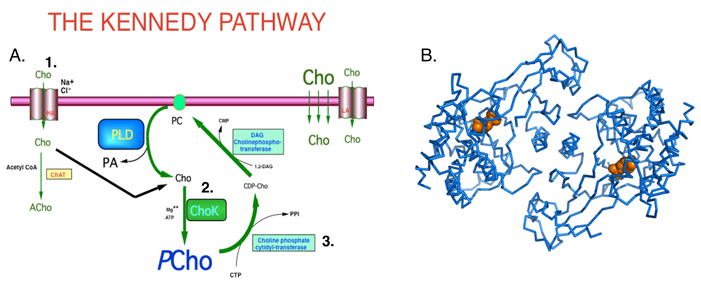
^{1}H-NMR (600 MHz) spectra of Il-4 (E-cad+) (top spectrum) and Il-4-E-cad- (bottom spectrum). The spectra show different peak ratios which suggest varying metabolite concentrations. *1* branched amino acids, *2a&2b* lactate, *3* alanine, *4* acetate, *5a* glutamine/glutamate, *5b* glutamate, *5c* glutamine, *6* dimethylamine, *7* creatine/creatine phosphate, *8* glycerophosphocholine/ phosphocholine, *9* myo-inositol, *10* glycine, *11* glutamine/glutamate/glutathione, *12* glucose, *13* NAD+, *14* aromatic amino acids.

Fig 3. Phosphocholine (PCho) and other statistically significant Il-4 & Il-4-E-cad- intracellular metabolites



Metabolite statistical significance determined via t-test and displayed as heat maps for both Il-4 and Il-4-E-cad-; red shows high relative intracellular concentrations, while blue shows low relative cellular concentrations (A). The red spectrum denotes Il-4-E-cad- and the blue spectrum denotes Il-4, and (B) shows an increase in Il-4-E-cad- phosphocholine (PCho) levels (p=0.001, FC=2).

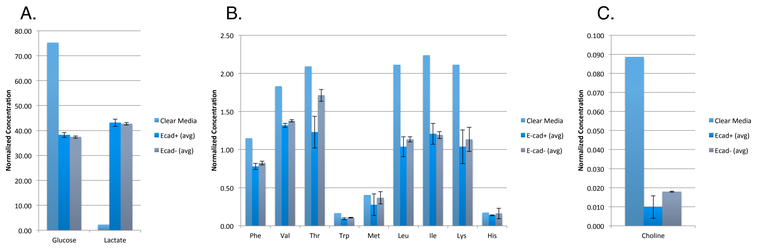
Fig 4. The Kennedy pathway and choline kinase



Phosphocholine is involved in the Kennedy Pathway (lipid membrane synthesis) and accumulation of PCho in Il-4-E-cad- may suggest: 1) increased choline uptake, 2) upregulation of choline kinase (ChoK), 3) downregulation of choline phosphate cytidyl-transferase (A).[2] Homodimeric structure of choline kinase with PCho (orange) in binding sites (B).[3] Upregulation of choline kinase has been implicated in a variety of malignant cancers.[4]

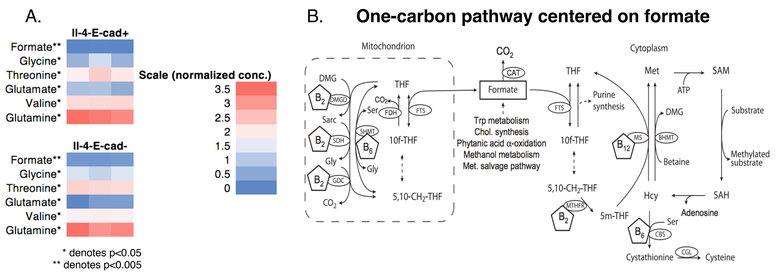
Results

Fig 5. Conditioned media analysis (extracellular metabolites)



Media concentrations of glucose are similar in both Il-4 and Il-4-E-cad-, which suggest same glucose uptake; lactate production is also similar (A). Media concentrations of essential amino acids also show similar uptake for both cell types with exception of threonine (B). Choline media concentrations are relatively similar for both cell types, thus phosphocholine accumulation may not be a result of increased choline uptake (C).

Fig 6. Statistically significant extracellular metabolites & potential link to one-carbon metabolism



Statistically significant metabolites displayed on heat maps: formate, glycine, threonine and valine levels increase in Il-4-E-cad- media, which suggests Il-4-E-cad- increased production of these metabolites (A). Accumulation of formate in Il-4-E-cad- could suggest altered one-carbon metabolism, since formate is a key intermediary metabolite. Methyl group transfers are vital to nucleotide and amino acid synthesis, among other pathways. Glycine is also implicated in these pathways as a byproduct of serine metabolism (B).[5]

Conclusions

- There are distinct metabolic differences between SCC cells that have lost cell-cell adhesion (Il-4-E-cad-) and SCC cells with cell-cell adhesion (Il-4-E-cad+).
- A possibly affected pathway is phospholipid synthesis and breakdown (Kennedy Pathway) due to accumulation of phosphocholine in Il-4-E-cad-.
- One-carbon transfers (methyl group) may also be affected due to increased production of glycine and formate.

Current & Future Directions

- Establish a direct link between phosphocholine accumulation and loss of cell-cell adhesion.
- Assess enzyme expression & activity - enzymes involved in choline metabolism and methyl group transfers.
- Chloroform/MeOH extraction to assess lipid concentrations of Il-4-E-cad- cells.
- **CURRENTLY**: observed Dab2 downregulation in tandem with E-cadherin loss. Assessing variable metabolic profiles of SCC based on degree of Dab2 loss.

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References

1. Images produced by S. Kamlarz, J. Reyes, E. Bingham & Dr. A. Alt-Holland (School of Dental Medicine). Used with permission.
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