Mass Spectrometry Profiling Reveals Markers of Response to Chemoradiation Therapy in Esophageal Cancer Patients

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Overview

Purpose

- Determine a proteomic signature that correlates with response to chemoradiation
- Determine if partial responders more closely resemble pCR or Non-responders

Methods

- Histology guided mass spectrometry profiling
- Statistical analysis and machine learning

Results

- 84% accuracy in leave-10%-out internal cross validation
- Greatest differences observed in normal-appearing tissue

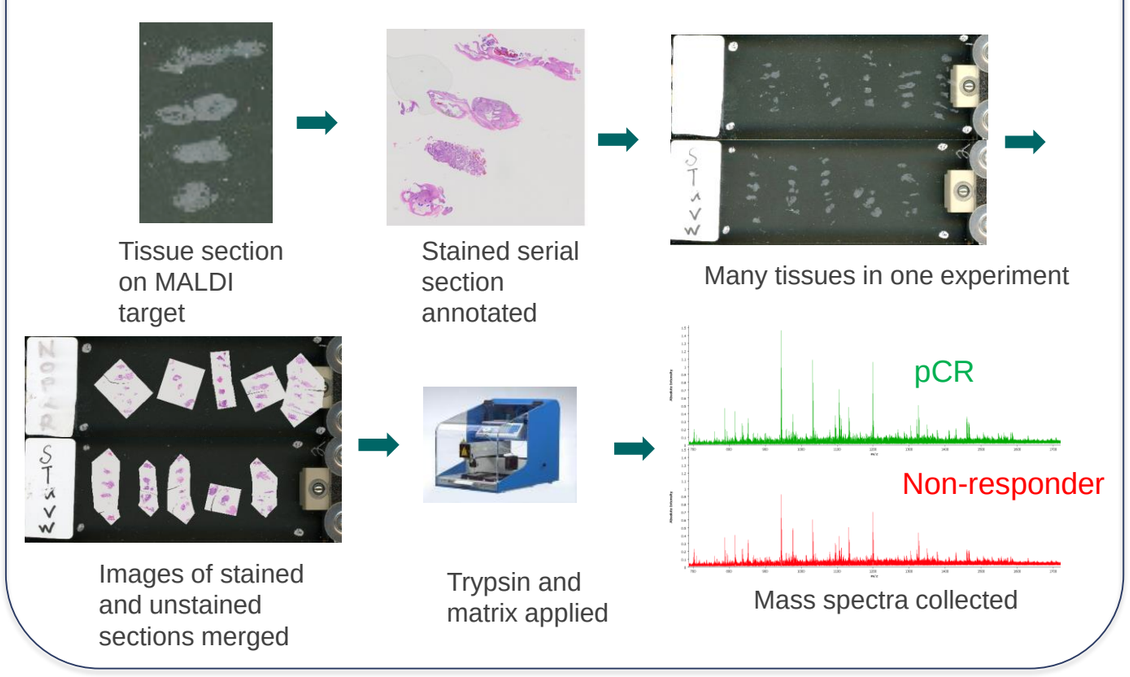
Introduction

For patients with locally advanced esophageal cancer, multimodal therapy consisting of concurrent chemoradiation (CRT) followed by surgery is the standard of care. Benefits to neoadjuvant CRT include tumor downstaging, and it is estimated that nearly one-third of patients undergo a complete pathologic response (pCR) which has been linked to improved overall survival. Predicting pathologic response to CRT could lead to tailored therapy; however, no accurate methods currently exist. Histology guided mass spectrometry profiling (HGMS) allows for targeted analysis of biomolecules in thin tissue sections from specific cells of interest. The objective of this study was to use HGMS to identify molecular signatures predictive of pathologic response to CRT in esophageal cancer.

Methods

- Pretreatment, FFPE biopsies from 47 esophageal cancer patients treated with concurrent CRT (50.4 Gy in 28 fractions) followed by surgery at ~8 weeks
- 5 µm thick sections collected onto ITO coated glass slides for HGMS or on regular glass slides for H&E staining
- Digital images of stained sections annotated with areas of tumor, stroma, and normal epithelium
- HGMS sections deparaffinized and antigen retrieved prior to on-tissue tryptic digestion and matrix application using SunChrom SunCollect robotic sprayer
- Mass spectra collected only from the annotated regions using Bruker ulftrafleXtreme MALDI TOF/TOF MS
- Statistical analysis and classification algorithm generation using SCiLS Lab 2017a

Histology Guided MS Workflow



Sample Summary

Cell Type	pCR 0% residual	Non-Responders >10% residual	Partial Responders <1%-10% residual
Cancer	27	12	8
Stroma	24	12	8
Normal Squamous Epithelium	19	6	5
Normal Gastric	3	5	2

Table 1. *Summary of number of samples having each histology and clinical outcome.*

Statistical Analysis

Cell Type	Peaks with Significant p-values	Peaks with area under ROC curve >0.8 or <0.2	LDA Internal Cross Validation Accuracy
Cancer	78	0	84.33%
Stroma	109	0	69.17%
Normal Squamous Epithelium	359	10	69.23%
Normal Gastric	82	24	

Table 2. *Statistical analysis results for comparisons between pCR and Non-responders for each histology. Both normal regions were combined to allow for enough statistical power for LDA.*

Significant Peptide Peak

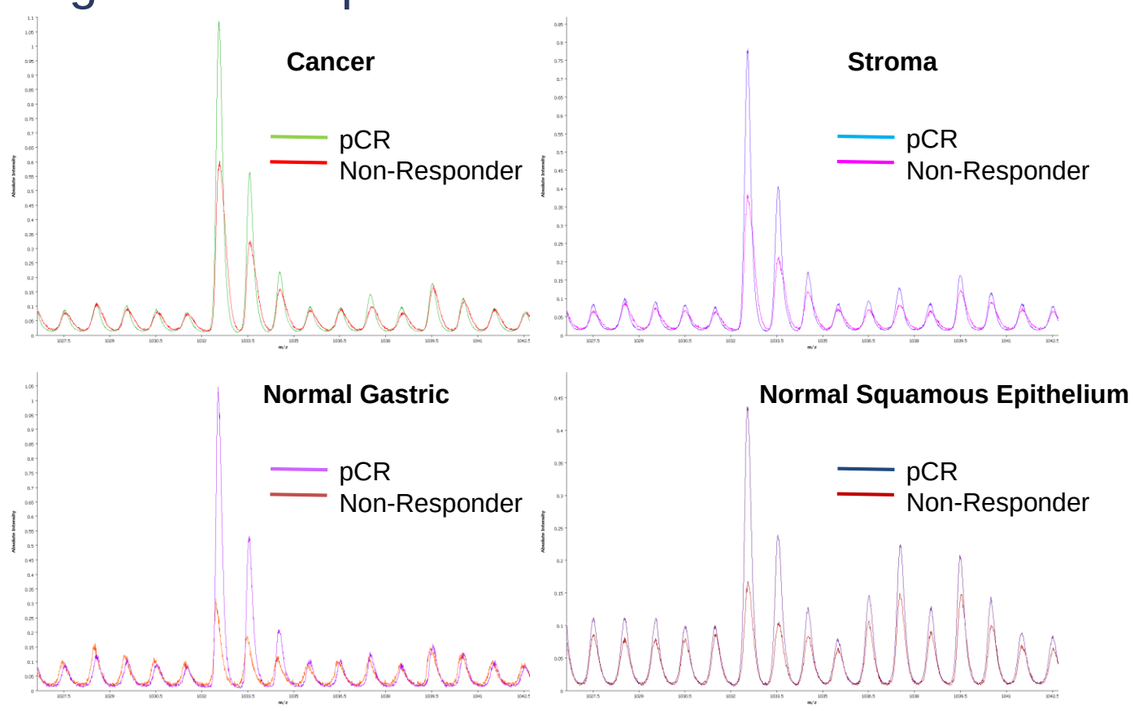


Figure 1*. Example of a peptide that was found to be significantly more abundant in pCR samples in all 4 histological regions studied. The peptide was identified as originating from histone H3.*

Classification of Partial Responders

Regions	Non-responders	pCR
D1 Stroma	0 (0.00%)	22 (100.00%)
A1 Stroma	0 (0.00%)	19 (100.00%)
X Stroma	4 (36.36%)	7 (63.64%)
P Stroma	0 (0.00%)	15 (100.00%)
U Stroma	5 (25.00%)	15 (75.00%)
Z Stroma	1 (5.88%)	16 (94.12%)
Y Stroma	18 (90.00%)	2 (10.00%)
L1 Stroma	2 (16.67%)	10 (83.33%)

Regions	Non-responders	pCR
D1 Cancer	5 (31.25%)	11 (68.75%)
X Cancer	1 (4.55%)	21 (95.45%)
Z Cancer	16 (76.19%)	5 (23.81%)
Y Cancer	6 (28.57%)	15 (71.43%)
L1 Cancer	3 (21.43%)	11 (78.57%)
A1 Cancer	2 (10.53%)	17 (89.47%)
U Cancer	6 (30.00%)	14 (70.00%)
P Cancer	2 (9.52%)	19 (90.48%)

Regions	Non-responders	pCR
D1 Normal Squamous Epithelium	14 (70.00%)	6 (30.00%)
D1 Normal Gastric	21 (84.00%)	4 (16.00%)
Z Normal Squamous Epithelium	12 (70.59%)	5 (29.41%)
A1 Normal Squamous Epithelium	6 (31.58%)	13 (68.42%)
Z Normal Gastric	7 (70.00%)	3 (30.00%)
U Normal squamous epithelium	2 (10.00%)	18 (90.00%)
L1 Normal Squamous Epithelium	1 (5.00%)	19 (95.00%)

Figure 2. *Results of classification of partial responders – number of spectra (% of total). Cancer and stroma favor pCR in most samples while the normal tissue favors Non-responders.*

Conclusions and Future Studies

- Significant differences observed between pCR and Non-responder patients
- Greatest number of significant peaks in normal appearing tissue
- Partial responders give mixed results when classified using LDA
- Additional samples to increase statistical power and validate results