Chemical Imaging of the Breast Tumor Microenvironment

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Statement of Purpose: Breast cancer is a heterogeneous malignancy that encompasses various morphological types and molecular abnormalities leading to a variety of clinical outcomes. Tumor metabolism is crucial in cancer onset and progression, and its causes and effects are under intense scrutiny. Standard clinical treatment for locally advanced breast cancer patients employs pre-surgical or neoadjuvant therapy. Defining the molecular and cellular mechanisms underlying tumor response or resistance to neoadjuvant therapy can aid in creating better strategies for treating patients.

Critical to achieve this project's objective are laboratory investigations and use of novel tumor analysis to elucidate pathways associated with tumor metabolic flexibility and chemoresistance. An important component of this project is analysis of frozen tissue samples taken pre- and post-neoadjuvant chemotherapy using time-of-flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS provides molecular information with imaging capabilities of subcellular spatial resolution within tissue samples. We use imaging ToF-SIMS and principal component analysis (PCA) to study thin sections of human breast tumors to help clarify links between fatty acid and amino acid composition within and around tumors and the potential drug resistance of these tumors. One sample of ductal carcinoma in situ (DCIS) and two paired pre- and post-chemotherapy treated breast tumors have been studied using PCA to determine molecular differences within tumor environments.

Methods: Data have been acquired on an IONTOF TOF.SIMS V using Bi_3^+ in both high mass and high spatial resolution modes. Multiple 1mm x 1mm areas per tissue slice were analyzed by stitching together 25 200µm x 200µm raster area scans in high mass resolution mode. Data was acquired in both positive and negative polarities. Ion dose per large area raster was $<5.0x10^{12}$ ions/cm^2. Spectral data was normalized to the sum of the peaks selected within the data set, which was subsequently square root transformed and then mean centered. PCA was then applied to the preprocessed data. Image data was Poisson scaled and mean centered before application of image PCA. Scores images generated by PCA that correlated with cellular and stromal areas were then used as masks to select regions of interest (ROI) that were reconstructed with ToF-SIMS software.

Results: High spatial resolution ToF-SIMS images give a chemical map of the tissue slices. Figure 1 shows an example of a ToF-SIMS image of a tissue biopsy showing DCIS tumor. The cell nuclei can be identified by following the sum of the CN- and CNO^- peaks. PCA of this sample (data not shown here) displays distinctly separate chemistries between highly cellularized tumor and the surrounding stromal regions within the tissue samples. Amino acid analysis of DCIS tumors using PCA indicates distinct chemical regions between the tumor interior, necrotic core and environment directly surrounding the tumor. Amino acid differences from PCA show higher intensity signals of valine, methionine and isoleucine within the tumor interior, while glycine, proline, and arginine are present surrounding the tumor.

Utilizing ROIs to select highly cellularized tumor regions within analysis areas followed by spectral PCA for two different sets of pre and post treatment tumor biopsies showed a near distinctive chemical separation between pre and post. Chemical differences can be seen between the pre and post treatment tissue biopsies relating to changes in fatty acids, monoacylglycerols, diacylglycerols and cholesterol. Biopsy samples pre-treatment showed higher loadings for vitamin E and oleic acid while post treatment samples had higher loadings for sphingomyelin and saturated fatty acids (stearic acid and palmitic acid).

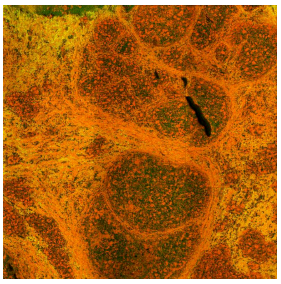

Figure 1. ToF-SIMS high spatial resolution image of ERRB2 tissue sample. Sum of CN^- and CNO^- (red) and sum of PO_2^- and PO_3^- (green) show the in situ tumors and their boundaries.

Conclusions: This study demonstrates the capability of ToF-SIMS to detect and identify metabolites present in complex tissue samples. PCA scores from image data sets can be applied to chemically analyze specific areas. Lipid saturation and cholesterol make large contributions to differences between pre/post treated cancer tissues in both cellular and stromal tissue regions. Spatial distributions of amino acids differ between in situ tumors, necrotic regions, and within the boundaries of the tumors.

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