Benign vs. Malignant Colorectal Polyps-Mass Spectrometry Diagnosis

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- In a previous experiment, the same two proteins, gi|119592539 hCG1787564 [Homo sapiens] Mass: 57590 and gi|119592490 hCG2040674 [Homo sapiens] Mass: 108178 were found in both colon adenocarcinoma and in adjacent normal tissue in the same patient.
- This was done with matrix assisted laser desorption ionization mass spectrometry imaging, MALDI (IMS) of biopsy tissue and liquid chromatography mass spectrometry (LCMS) on the extract from a contiguous tissue section.

- Are these proteins evidence of malignancy, and does the use of histopathology alone underestimate the extent of potential malignant disease?
- We hypothesized that histopathology in combination with IMS, can identify malignant disease in histologically non-malignant or benign appearing polyps.

Colorectal Cancer

- The third most common cancer in U.S.
- The second deadliest cancer, 49,960 deaths annually.
- More than 1 million Americans living with colorectal cancer.
- 148,810 new cases of colorectal cancer were diagnosed in 2008, including
  - 108,070 cases of colon cancer
  - 40,740 cases of cancer of the rectum

Centers for Disease Control and Prevention (CDC). *MMWR Morb Mortal Wkly Rep* 2008; **57**: 253.

Polyps (Adenomas)

- Definition: Adenomas are dysplastic and have malignant potential.
- The prevalence of adenomas is about 25-30% at age 50, Autopsy studies rates as high as 50% by age 70.
- The time for development of adenomas to cancer is about 7 years (5-10).
- Nearly all CRCs arise from adenomas, but only 5-10% progress to cancer.
- Three variants: Tubular (75-87%), tubulovillous (8-15%), Villous (5-10%).

Methods

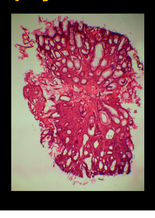
- Specimens obtained from individuals with colon adencarcinoma (polyp 5) and compared to normal colon tissue (polyps1-4).
- Endoscopic colon polyp biopsies immersed in 3.7% fresh paraformaldehyde solution for one hour.
- The tissue was cryoprotected by immersion in 30% glucose.
- Freezer artifact free 1 µm thick cryosections, were obtained for histology and IMS.

Methods

- Mirror images of the contiguous sections were obtained for histology, and stained with hematoxylin and eosin, H&E.
- Tissue corrosion was avoided by sublimation of MALDI matrix onto the tissue. No solvent (TFA) was used.
- IMS on 5 individual polyps, images 1-12, were displayed on spectra and imaged with Biomap (imaging software).
- The laser spot interval was reduced from 250 µm in images 11 and 12 to 30 µm in images 1-10. This produced a finer raster and higher image resolution with greater conspicuity of the image densities.

Pevsner PH, Melamed J, Remsen T, Kogus A, Francois F, Kessler P, Stern A, Anand S. *Biomarkers Med* 2009; **3**: 55.

Colorectal Polyp Endoscopic Biopsy-H&E Stain



Colorectal Polyp Endoscopic Biopsy Sublimated Sinapic Matrix

Results

- Additional proteins were identified in the colon biopsies using Mascot search engine interrogation of the NCBInr and Swiss-Prot databases.
- IMS displayed the loci of proteins in these histologically normal polyps.
- The proteins listed at the top of the MALDI images are represented by the red color in the images.

Polyp 1 3024.09 Da Envelope Glycoprotein [HIV-1](red in image)

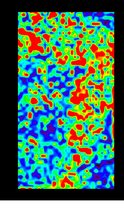


Image 1

Polyp 1 38390.3 Da Homo- Sapiens Carbonic Anhydase X II (red in image)

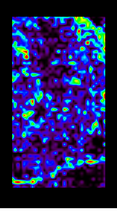


Image 2

Zinc-containing enzymes that catalyze the reversible hydration of CO2 in a 2 step mechanism

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Polyp1 43441.5 Da Sirtuin 3 Isoform A (red in image)

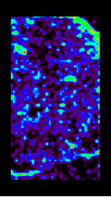

Image 3

Stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70

Polyp 1 38390.3 Da Homo- Sapiens Carbonic Anhydase X II (red in image)

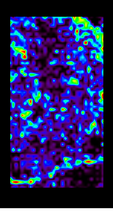

Image 3a

Zinc-containing enzymes that catalyze the reversible hydration of CO2 in a 2 step mechanism

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Polyp 2 3024.09 Da Envelope Glycoprotein [HIV-1] (red in image)

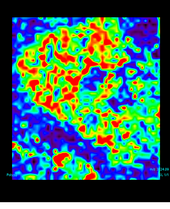

Image 4

Polyp 2 10143.0 Da Immunoglobulin Heavy Chain Variable Region (red in image)

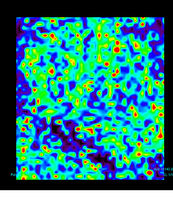

Image 5

Polyp 2 21395.7 Da Transmembrane Protein 126A (red in image)

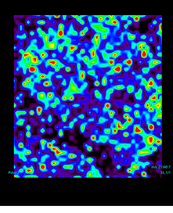

Image 6

Polyp 2 46426.0 Da Polyprotein [Dengue Virus Type 1] (red in image)

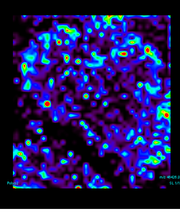

Image 7

Polyp 2 53545.7 Da Transcription Factor 7-Like 2 (T-Cell Specific, HMG-Box) Isoform, CRA_a (red in image)

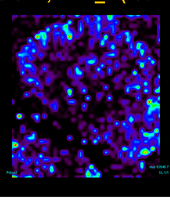

Image 8

Binds to minor groove of DNA in a highly sequence-specific manner

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Polyp 2 67324.1 Da Ras Homolog Gene Family, Member T2, Isoform (red in image)

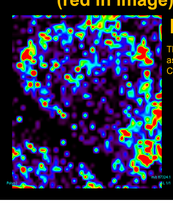

Image 9

This protein in known to be associated with Colon Cancer

Polyp 3 10746.1 Da NADH-Ubiquinone Oxidoreductase Chain 1 (red in image)

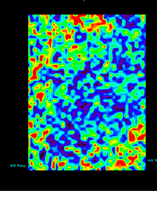

Image 10

NADH-ubiquinone oxidoreductase chain 1, a known colon cancer protein

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Polyp 4 6139.5 Da Transmembrane Tyrosine Kinase Receptor (red in image)

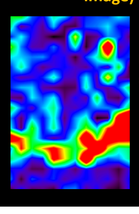

Image 11

Up regulated in colon cancer

Polyp 5 7740.21 Da hCG2040855 (red in image)

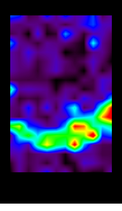

Image 12

Present in 25% of colon cancers and associated with higher mortality

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Discussion

- Proteins in images 2-3 (polyp 1) and 5-6 (polyp 2), were normal.
- Images 1 (polyp 1) and 4 (polyp 2), had Envelope Glycoprotein [HIV-1]
- Image 9 (polyp 2), had Ras homolog gene family, member T2, isoform, a known colon cancer protein was identified.
- NADH-ubiquinone oxidoreductase chain 1, a known colon cancer protein was identified in image 10, (polyp 3).

– Habano W, Sugai T, Yoshida T, Nakamura S. *Int J Cancer* 1999; **83**: 625.

Discussion

- Transmembrane tyrosine kinase receptor, a protein up regulated in colon cancer was identified in image 11, (polyp 4).
- HCG2040855, a protein present in 25% of colon cancers and associated with higher mortality was identified was identified in image 12, (polyp 5).
- Putative colon cancer proteins were identified in 3 of the 4 histologically non-malignant polyps.

Conclusion a

- Putative carcinoma proteins were again identified in histologically non-malignant polyps. However, only 1/6 sections from polyp 2, image 9, demonstrated a protein associated with colon cancer.
- In our previous report, we showed that IMS images can identify protein biomarkers of putative carcinoma proteins in histologically non-malignant polyps.

Conclusion b

- In this report, we refined the images with a finer raster and demonstrated that multiple sections must be obtained to exclude colon cancer proteins.
- These findings suggest a new role for MALDI imaging in diagnostic pathology and confirm our previous findings. These findings support our hypothesis that IMS can identify tumor proteins, in histologically non-malignant polyps.

Improvements For Further Investigations

- Multiple IMS images must be obtained from each sample for thorough examination of the tissue for colon cancer proteins, a single image could miss a colon cancer protein.
- Confirm MALDI Proteins
  - Perform protein extraction on contiguous sections.
  - Study extracts of multiple sections of each lesion with ultra high sampling speed LCMS to confirm protein identification as in our colon polyp study.