Towards a future of personalized cancer care for glioblastoma patients through development and implementation of novel molecular diagnostic tools

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The David Geffen UCLA School of Medicine
President-elect; the American Society for Clinical Investigation
"As more drugs that target specific components of signal transduction pathways become available and as we increase our knowledge of the complexity of these signaling networks, the burden of selecting the right drug combinations for each individual cancer patient will ultimately shift to the pathologist who must identify the underlying defect in each tumor."

Shaw and Cantley, Nature 2006
Whether through clonal evolution or failure to eradicate a stem cell compartment, or both, cellular and molecular heterogeneity are central to therapeutic resistance in cancer patients!
Highly trans-disciplinary effort created, funded and supported through the NCI's Centers of Cancer Nanotechnology Excellence Program – already implemented and poised to alter care in the clinic

UCLA - Mischel group:
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CIT: Heath Group
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Ophir Vermesh
Udi Vermesh

Other collaborators: UCLA - Raphael Levine and Francoise Remacle
ISB - Lee Hood Group
We developed a technology called DEAL that allows for the separation of a very small tumor section into well-defined cell populations for subsequent molecular analysis.

A cancerous mass contains a heterogeneous mixture of tumor cells expressing different cell surface markers, interspersed immune cells such as lymphocytes, monocytes and microglia, and supporting elements such as vascular endothelial cells.
DEAL technology (Bailey et al., 2007; Kwong et al, 2009):

1. Highly multiplexed protein quantification - DEAL biobarcode assays
2. Capture of defined cell types from tumor samples - DEAL cell sorting arrays

Highly specific capture - 98% capture specificity in a mixed population with as few as 1000 cells
DEAL allows for the separation of a very small tumor section into well-defined cell populations for in-depth molecular analysis.
Capture of EGFR expressing (EGFR-amplified) tumor cells directly from a clinical tumor sample
Transcriptome analysis from defined tumor cell subpopulations captured from a GBM patient sample

RNA extraction and real-time PCR

Real time RT-PCR of cells captured on DEAL arrays using αEGFR, αCD31 and αCD45 antibodies

mRNA expression
Global analysis of DNA copy number in defined tumor cell subpopulations

Operating Room
Tumor biopsy
Collagenase treatment
Formalin fixed & paraffin embedded

FISH
Immunohistochemistry

Chromosomal gains or losses (e.g. EGFR amplification, polysomy of chromosome 7)

EGFR positive, etc…

Label DNA with Cy-3
(EGFR and EGFR/vIII DNA)

Extract genomic DNA from both arrays for genomic amplification

Label DNA with Cy-5
(CD45 and CD31 DNA)

Hybridize to Agilent CGH array

Collaboration with Dr. Stanley Nelson
Global analysis of DNA copy number and targeted sequence analysis of defined tumor cell subpopulations captured on DEAL arrays
Co-activation of all three GBM “core pathways” in a tumor subpopulation defined by EGFR expression

Adapted from TCGA Nature (2008)
Co-activation of three GBM “core pathways” in a tumor subpopulation defined by EGFR expression in multiple GBM patients

Summary to date:

• Captured defined cell populations from 80 clinical samples to date

• Performed in-depth molecular analysis of DNA copy number alterations, mutations in “core pathway” genes and transcriptional analysis of a subset

• Clear evidence of multiple genetic lesions in each of the core pathways in all of the samples studied in-depth
DEAL can detect rare tumor cells in histologically “normal” tumor margins that cannot be detected by pathological examination and molecular analysis of tumor tissue.
Extending the technology to study networks at the single cell level

Laser capture microdissection of a single EGFR+ cell a single CD31+ vascular endothelial (both captured on DEAL arrays).

DNA Amplification

CD31+ (vascular vs Tumor Cells Endothelial cells) EGFR+

Population analysis – Analysis of DNA copy # alterations between tumor cell subpopulations – aCGH of EGFR versus CD31 captured cells.

Single cell analysis – cells were laser capture microdissected from DEAL captured population of EGFR and CD31 captured cells. After whole genome amplification, SNP analysis demonstrates a pattern of copy # alterations that are similar to those identified in the defined cell populations. (done with Dr. Lynda Chin)
Single cell proteomics linking DEAL-based tumor cell capture with multiparameter quantitative proteomic measurement.
Quantitative measurement of signal transduction at the single cell level

Summary:

• Cytoplasmic signaling proteins and secreted proteins monitored under influence of biological, chemical or physical perturbations

• Resolution to the single cell level

• Information theory integrates measurements to produce a robust and predictive network connecting signal transduction pathways with genetic alterations (i.e. information from TCGA) and with blood chip diagnostics
Non-invasive, real-time monitoring of response to targeted therapy in GBM patients in the clinic

Goal: Non-invasive “real-time” monitoring of response to targeted therapy - Avastin as test case
**U87 GBM Cell line - response of protein secretion profile to drugs for 12 proteins (GBM cells 'look' like immune cells here)**

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<th>IL-6</th>
<th>G-CSF</th>
<th>MIF</th>
<th>MMP3</th>
<th>VEGF</th>
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*Data is contrast enhanced - many changes are very low amplitude*

Young Shik Shin
Tiffany Huang
P Mischel
J Heath
2009
Best 14 proteins

Best 21 proteins

• Cluster 1 - 7/7 (100%) of patients have tumor growth during avastin therapy

• Clusters 2, 3 - 6/31 (19%) patients have tumor growth during avastin therapy

• P < 0.0001
DEAL to capture and facilitate single cell genomics of clinical samples

Integrated barcode chips for single cell proteomics

Blood barcode chips to monitor response to avastin in GBM patients

Heterogeneous tumor

Sort into defined populations by cell surface markers

New Technology

Multiplexed proteomics (secreted and cytoplasmic) of single cells, small cell Colonies & model GBM micro-environments. Signaling networks elucidated from perturbative experiments & analysis.

Multiplexed analysis at
- Genome
- mRNA
- Protein levels

85 patient tumors analyzed to date

Several very large data sets collected & partially analyzed to date

Overview of the UCLA/Caltech program

>300 patient bloods collected & analyzed to date

Stratifying GBM patient responders & non-responders to therapies; Initial application is for Avastin

New Technology
Thank you!