Multiplex Analysis of Intracellular Signaling Pathways in Lung Cancer FNA Samples by Microbead Suspension Arrays

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Molecular targeted therapy for NSCLC

- Treatment is designed to stop the action of abnormal proteins that cause cells to grow and divide out of control while minimizing damage to healthy cells.

- Molecular Targeted Cancer Therapies will work:
  - In tumors in which the target is relevant
  - When the target is properly inhibited
Challenges in targeted therapy

Targeted therapeutics induce clinical responses in only a limited number of patients where the target is important.
How to identify the patient population with the target?

Prediction of tumor response *ex vivo* to:
- Optimize the selection of therapeutic agents according to the tumor's sensitivity to a drug prior to initiation of therapy.
Tumor Fine Needle Aspiration Biopsy is an Effective Tool to Predict and Assess Tumor Response to Therapy

- Fine Needle Aspiration (FNA) technique is a minimally invasive, cost effective and reliable routine diagnostic procedure to diagnose neoplastic lesions.
- FNA yields enriched viable tumor cell populations.
- FNA is suitable for serial sampling over the course of treatment to monitor therapy effect \textit{in vivo}.
EGFR – A good target for NSCLC

- High level of receptor expression compared with healthy tissue
- EGFR - Key role in tumor cell growth & function
- EGFR inhibitors have no severe toxicity
Human Epidermal Growth Factor Receptor Family

EGF, TGFα, β Cellulin
Amphiregulin, HB-EGF

No specific ligands - often acts as dimer partner

Heregulins
NRG2
NRG3
Heregulins
β-cellulin

erbB1
HER1
EGFR

erbB2
HER2
neu

erbB3
HER3

erbB4
HER4
EGFR – A good target for NSCLC
Strategies to inhibit EGFR signaling

EGFR tyrosine kinase inhibitors

Anti-EGFR mAbs

ATP
Potential mechanisms of resistance to EGFR inhibitors

- Resistance mutations
- Gain-of-function mutations in downstream signalling molecules
- Loss or inactivation of signal downregulator

- Activation of alternative pathways (HER3, MET, IGFR)
Patient selection for EGFR TKIs

Positive selection
- Patient characteristics
  - never-smoking, female, adenocarcinoma, East Asian
- EGFR mutations
- EGFR FISH

Negative selection
- RAS mutations
- Resistant EGFR mutations (e.g. T790M)
- C-Met amplification
How to identify effective therapeutic opportunities tailored to the individual patient?
FNA-based *ex vivo* and *in vivo* assays to predict and assess the efficacy of targeted drugs

- Cancer cells obtained by FNA can be used to test *ex vivo* the effects of targeted anticancer agents

- The *ex vivo* effects correlate with antitumor activity in vivo

Hidalgo et al, Mol Cancer Ther. 2006; 5: 1895-903
Rubio-Viqueira et al, Cancer Ther. 2007; 6:515-23
Altiok et al, 2008, submitted for publication
Beadlyte multiplex assays

A highly sensitive and quantitative molecular technique for assessment of drug response in small tumor samples

Simultaneous analysis of the activity of multiple intracellular signaling proteins in small tumor FNA samples

(1-5 microgram total protein)
Many Beadlyte® Kits are Bead-Based Sandwich Immunoassays

Bead with capture antibody

Capture antibody binds analyte

Fluorescence labelled reporter antibody binds to capture analyte

Bead ID and reporter quantity determined by laser detector
CASE STUDY

Patient 1
T.D. 39 yo WM

Patient 2
J.R. 54 yo WM

No EGFR or RAS mutations

Question: Which of the following drugs will likely be effective?

A) Erlotinib (EGFR-i)
B) CI-1040 (MEK/ERK-i)
C) Dasatinib (SRC-i)
D) None of the above
A drug sensitivity assay to predict tumor response to targeted therapeutics
Question: Which of the following drugs will likely be effective?

A) Erlotinib (EGFR-i)
B) CI-1040 (MEK/ERK-i)
C) Dasatinib (SRC-i)
D) None of the above
Patient 2

The image shows a graph with the following labels:

- **P-EGFR**
- **P-ERK**
- **P-STAT3**
- **P-AKT**

The x-axis represents different treatments:

- Control
- CI-1040
- Dasatinib
- Erlotinib

The y-axis represents the levels of phosphorylation, with values ranging from 0 to 45.

The graph illustrates the phosphorylation levels in different samples treated with the indicated drugs.
Question: Which of the following drugs will likely be effective?

A) Erlotinib (EGFR-i)  
B) CI-1040 (MEK/ERK-i)  
C) Dasatinib (SRC-i)  
D) None of the above
Conclusions

The FNA-based drug sensitivity assays are rapid, highly sensitive and cost effective.

The implementation of these approaches may allow tailoring of the most efficient therapeutic regimen for individual patients.
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