



LI-COR® Biosciences

July 14, 2010

Upcoming webinars: [register here](#)

Part 3. *In vivo* cancer imaging: NIR optical probes

Sept. 23, 2010; registration begins in early September

Part 4. *In vivo* cancer imaging: custom probes

Part 5. *In vivo* imaging: implications for translational research

On-demand webinar videos:

Part 1. Introduction to near-infrared (NIR) fluorescent methods

[Watch now](#)

Part 2. Quantitative protein analysis in cancer research

[Available soon](#)

1. Why quantitative protein analysis?
 - a. New biological problem - qualitative answers very useful
 - b. Quantitative answers can enhance understanding
 - i. Protein level
 - ii. Number of protein molecules
 - iii. Enzyme activity
 - iv. Feasible with IR fluorescent imaging

2. Cell characteristics
 - a. Normal cells
 - i. Require growth factors
 - ii. Anchorage dependence
 - iii. Contact inhibition
 - iv. Limited cell proliferation
 - b. Cancer cells
 - i. [Hanahan and Weinberg. Cell 100: 57 \(2000\)](#)
 - ii. self-sufficiency in growth signals
 - iii. insensitivity to anti-growth signals
 - iv. invasion and metastasis
 - v. limitless cell division
 - vi. angiogenesis
 - vii. evasion of apoptosis

3. Effects of Rb on Cyclin D3 protein stability
 - a. [DeSanta et al. Mol Cell Biol 27:7248 \(2007\)](#)
 - b. Half-life of Cyclin D3

- i. Proliferating cells: 20 min
 - ii. Differentiated cells: 50 min
 - iii. Rb-deficient cells: 13 min
 - c. T283A mutation
 - i. Cyclin D3 very stable in proliferating and differentiated cells
 - ii. Stable even in Rb-deficient cells
- 4. Expression of M1 isoform of pyruvate kinase
 - a. [Clower et al. Proc Natl Acad Sci 107:1894–1899 \(2010\)](#)
 - b. M1 – primarily in differentiated cells
 - c. M2 – in proliferating and cancer cells
 - i. Images: [Molecular & Cellular Proteomics 4:887–901 \(2005\)](#)
 - d. Expression of certain RNA splicing repressors much higher in proliferating cells
 - ii. Knock down hnRNP A1 and hnRNP A2
 - 1. M1 abundance increases 6-fold
 - ii. Knock down PTB
 - 1. M1 abundance increases 3-fold
- 5. Role of EGF receptor in cellular response to *Helicobacter pylori* infection
 - a. [Y. Du et al. / Microbes and Infection 9 \(2007\) 838-846](#)
 - b. *H. pylori* activates EGFR signaling in gastric epithelial cells
 - c. In-Cell Western™ assay
 - i. Co-culture with *H. pylori*
 - ii. Fix and permeabilize
 - iii. Stain with antibodies to unmodified ERK and p-ERK
 - d. Effect of EKB-569 inhibitor on ERK signaling
 - i. Blocks ERK response to EGF
 - ii. Partially blocks ERK response to *H. pylori* infection
 - 1. Confirms involvement of EGF receptor signaling
- 6. Regulation of p53
 - a. [Wang et al. PNAS 104: 12365–12370 \(2007\)](#)
 - b. Hdmx and Hdm2
 - i. Negative regulators of p53
 - ii. Study molar relationships between p53, Hdm2, and Hdmx
 - c. Absolute quantification with fluorescent Westerns
 - i. Generate standard curves with purified proteins
 - ii. Analyze cell lysates and calculate number of protein molecules per cell
 - iii. Levels of all 3 proteins is variable in normal and tumor cells
 - d. Stoichiometry of regulation after NCS treatment
 - i. Normal cells
 - 1. Hdm2 increases

- 2. Hdmx decreases
 - 3. P53 increases, and p53-to-Hdmx molar ratio increases
 - ii. Tumor cells
 - 1. Hdm2 increases
 - 2. Hdmx decreases, but is still high
 - 3. P53 increases, but remains submolar to Hdmx
7. MicroWestern Array analysis of EGF-induced signaling
- a. [Ciaccio et al. Nature Methods 7:148 - 155 \(2010\)](#)
 - b. Study timing and amplitude of phosphorylation dynamics in EGF response
 - c. Method
 - i. [video description of technique](#)
 - ii. Treat cells with EGF
 - iii. Array cell lysates on gel
 - iv. Semi-dry electrophoresis and membrane transfer
 - v. Probe with 192 antibody combinations
 - 1. 96-well gasket apparatus
 - 2. Two-color IR fluorescence
 - d. Very small sample size
 - i. Microwestern array: 10^3 cells
 - ii. Mass spectrometry: 10^8 cells
 - iii. Western blot: 10^5 cells
 - e. Results
 - i. Quantified 91 phosphosites from 67 proteins
 - ii. Six time points
 - iii. 10,000 signaling observations
 - iv. Detected phosphosites overlooked by previous mass spec studies
 - f. Computational analysis
 - i. Grouped in 5 clusters, based on timing of phosphorylation response
 - 1. Earliest response: receptor tyrosine kinases; membrane-associated proteins
 - 2. Mid-response: kinases and transcription factors; cell-cycle-related kinases and substrates
 - 3. Late response: Akt, p38
8. Using computational modeling to predict off-target effects of kinase inhibitors on cell behavior
- a. [Kumar et al. Mol Pharm 73:1668 \(2008\)](#)
 - b. Method
 - i. Stimulate cells with EGF or HRG.
 - ii. Kinase inhibitors (PI3 kinase, MEK, or the EGFR)
 - iii. Quantify phosphorylation of ERK, p38, Akt, and EGFR
 - iv. In-Cell Western for increased throughput
 - c. PI3 kinase inhibitor (LY294002)

- i. Reduces migration of HRG-stimulated cells
 - ii. Does not reduce migration of EGF-stimulated cells
 - iii. Phospho-Akt does not predict cell migration
- d. Computational modeling
 - i. Need to analyze off-target modulation of p38, ERK, and EGFR
 - ii. Model can now predict migration behavior

9. Cortactin and MMP activity

- a. [Clark et al. Canc Res 67:4227 \(2007\)](#)
- b. Cortactin expression is associated with tumor invasiveness
- c. Gelatin zymography method
 - i. Cast SDS-PAGE gel with 0.25% gelatin
 - ii. Run protein samples
 - iii. Incubate 37C
 - 1. Proteases digest embedded gelatin
 - iv. Coomassie stain
 - 1. Protease activity appears as clear zones in stained gel
 - v. RNAi knockdown of cortactin
 - 1. Reduced MMP-2 and MMP-9 secretion

10. VE-cadherin and the VEGF response

- a. [Ha et al. J Biol Chem 283:7261 \(2008\)](#)
- b. VE-cadherin inhibits mitogenic signaling through ERK
- c. Control cells
 - i. EGF treatment induces Src phosphorylation
- d. VE-cadherin RNAi knockdown cells
 - i. Src phosphorylation reduced
 - ii. ERK phosphorylation enhanced

11. Quantitative protein analysis important for understanding of protein function

- a. Odyssey® Infrared Imaging System
- b. [Odyssey Fc Imaging System](#)
 - i. IR fluorescence
 - ii. Chemiluminescence

12. Upcoming sessions of the Cancer Research Webinar Series

- a. Part 3: *In vivo* molecular imaging with NIR optical probes
 - i. September 2010
 - ii. receptor targeting
 - iii. structural imaging of bone
 - iv. imaging of vasculature and lymphatics
- b. Part 4: *In vivo* imaging with custom probes

- i. Development and validation of an optical probe
- c. Part 5: *In vivo* imaging – implications for translational research
- d. [Request a registration reminder](#)