The use of nanoimmunoassay (NIA) technology to predict response to insulin-like growth factor-1 receptor (IGF1R) inhibition in head and neck squamous cell carcinoma (HNSCC)

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Abstract #1248

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Background

Signaling from the IGF1R plays a role in resistance to anti-cancer therapy in HNSCC.[1] Thus, targeted inhibition of the IGF1R holds substantial therapeutic potential. While several inhibitors of the IGF1R are in clinical trials, there is no biomarker that predicts tumor responsiveness to anti-IGF1R therapy. Such a predictive biomarker is likely to be a component of the most prominent downstream signaling cascades from the IGF1R, which include the MEK/ERK or PI3K/AKT pathways that principally regulate proliferation and survival, respectively.

Hypothesis

Short-term changes in the activation status of downstream signaling proteins will be predictive of long-term tumor response to inhibitors of the IGF1R, and these changes will be detectable in minimal tissue samples using NIA technology.

Objectives

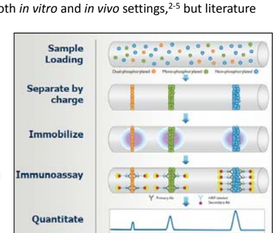
(1) Investigate the use of NIA technology to quantify markers of growth inhibition and apoptosis which can ultimately be used in preclinical and clinical studies.

(2) Identify short-term predictors of long-term growth inhibition and apoptosis when the IGF1R inhibitor OSI-906 is used alone or in combination with other therapeutic agents in HNSCC cell lines.

Nanoimmunoassay

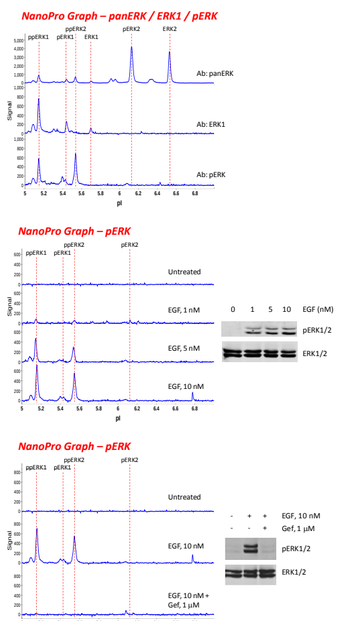
NIA uses capillary isoelectric focusing (IEF) and immunodetection to characterize and quantify proteins in extremely small samples (200 nL; see scheme below). NIA can resolve different phosphoisoforms of a given protein and these can be detected simultaneously with a single antibody. Recent papers have demonstrated NIA-based detection of a variety of proteins in both *in vitro* and *in vivo* settings,[2-5] but literature on this approach remains limited.

Clinically, NIA allows for assessment of the activation state of signaling proteins in small biopsies such as fine needle aspirates (FNAs). ERK has been detected using NIA in as few as 25 cells.[3] In the present study, we used NIA to evaluate ERK, PARP and BIM levels in HNSCC cells *in vitro* and *in vivo*. NIA analyses were performed using the ProteinSimple NanoPro 100, which simultaneously analyzes and automatically quantifies 12 unique samples with up to 12 unique antibodies.



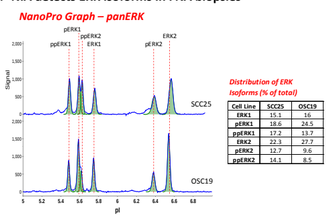
Results

1. NIA detects all six non-phosphorylated, mono-phosphorylated, and di-phosphorylated ERK1 and ERK2 isoforms in HNSCC cell lysates



Cal27 cells, treated as indicated for 15 min. Whole cell lysates were prepared and subjected to NIA and immunoblot analysis with the primary antibodies indicated. Gef = gefitinib

2. NIA detects ERK isoforms in FNA biopsies

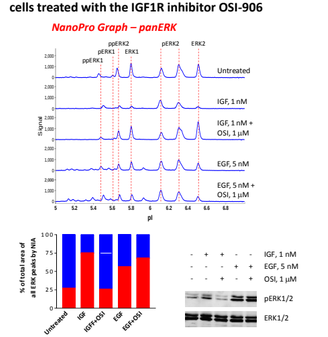


Distribution of ERK Isoforms (% of total)

Cell Line	SCC25	OSC19
ERK1	15.1	16
pERK1	18.6	24.5
ppERK1	17.2	13.7
ERK2	22.3	27.7
pERK2	12.7	9.6
ppERK2	14.1	8.5

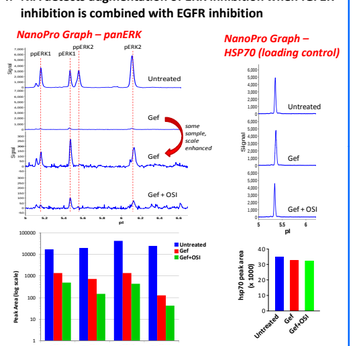
Subcutaneous flank xenografts were generated in nude mice from SCC25 and OSC19 cells and were biopsied by standard FNA technique. FNA specimens were lysed and subjected to NIA using the panERK antibody. Automated peak quantification and distribution were performed by the NanoPro software.

3. NIA detects changes in ERK phosphorylation in HNSCC cells treated with the IGF1R inhibitor OSI-906



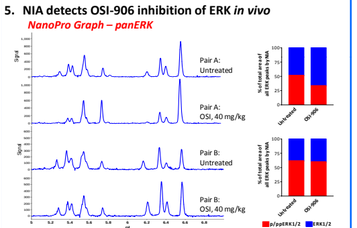
Cal27 cells were pretreated with OSI-906 (OSI) as indicated for 2 h followed by stimulation with IGF or EGF for 15 min. Whole cell lysates were subjected to NIA and immunoblot analysis with the panERK antibody. IGF = des[1-3]IGF-1

4. NIA detects augmentation of ERK inhibition when IGF1R inhibition is combined with EGFR inhibition



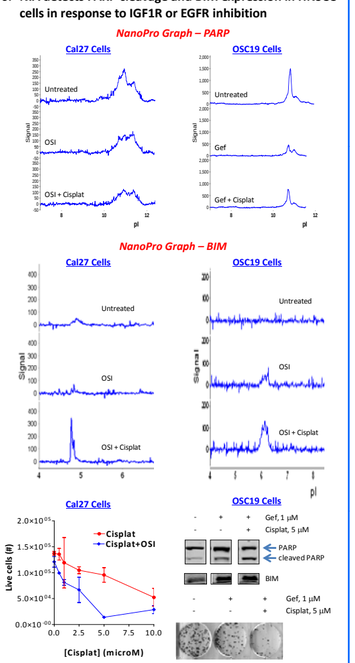
SCC25 cells were treated with 1 μM gefitinib (Gef) ± 1 μM OSI-906 (OSI) for 2 h then subjected to NIA using a pERK antibody (left). Peak areas are graphed using a logarithmic scale to show the sensitivity range. The same samples were assessed using an HSP70 antibody (right) to demonstrate equivalent protein content.

5. NIA detects OSI-906 inhibition of ERK *in vivo*



Two pairs of OSC19 xenografts were treated with 2 daily doses of vehicle or OSI-906 (OSI). Tumors were assessed by NIA for ERK phosphorylation which is summarized graphically demonstrating differential molecular response.

6. NIA detects PARP cleavage and BIM expression in HNSCC cells in response to IGF1R or EGFR inhibition



Cal27 or OSC19 cells were treated with OSI-906 (OSI) or gefitinib (Gef) ± cisplatinum (Cisplat) as indicated for 24 h. Cell lysates were assessed by NIA and immunoblot for PARP and BIM. Cal27 cells were counted and a dose-response curve was plotted. OSC19 cells were subjected to clonogenic assay.

Summary

- NIA can quantify the degree of ERK activation in HNSCC in vitro and in vivo using samples as small as a FNA.
- ERK activation can be quantified over a range greater than 2 orders of magnitude, and augmentation of ERK inhibition with gefitinib can be observed in HNSCC cells when gefitinib is combined with the IGF1R antagonist OSI-906.
- Under different conditions, similar tumors exhibit different reductions in ERK activity in response to IGF1R inhibition within 48 hrs, suggesting that ERK may have utility as an early predictor of response to IGF1R antagonists. Additional studies will focus on defining a correlation between change in ERK activity and cell/tumor response.
- The apoptotic markers PARP and BIM can be detected by NIA and their behavior on NIA analysis correlates with reduction in cell number and reduced clonogenic survival. Future work will further validate these markers in the NanoPro and correlate their levels with cell/tumor response.

References

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Acknowledgments

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