Multiplexing technology for in situ Small-Cell Lung Cancer (NSCLC)

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BACKGROUND

NSCLC is a heterogeneous neoplasm comprising several histologic types, ecologies, genetics, survival and response to therapy. The number of potential molecular targets each subtype has increased dramatically. While genomic tests can provide relevant quantitative multiple biomarker and response to therapy. The number of potentially relevant biomarkers in each subtype has increased dramatically. While genomic tests can provide relevant quantitative multiple biomarker detection and minimally invasive procedures. A recent survey of US pathologists presented at ASCP 2013 meeting, concluded excellent sample availability is a big hit of NSCLC samples recently handled by these pathologists. However, subcellular localization of marker expression linked to tumor histobiology necessitates methodological refinement. With the development of a new platform, MultiOmyx™ that allows in situ, multiplexed subcellular analysis of over 60 proteins, this study aims to demonstrate the feasibility of detailed in situ molecular profiling and perform comparative analysis of common cancer pathway and prognostic markers on a single tissue section.

METHODS

Multiple immunofluorescence staining and imaging of over 60 biomarkers (Table 1) including several cell-surface antigens, select members of RTK, MAPK, NFkB, and JAK/STAT pathways, for example, Her2, EGFR, pEGFR, cMET, pCmet, EGFR and cMET positivity in AD (Table 3). Data were filtered to remove patients with severe over-segmentation or false positive detection over non-specific signals. Image analysis was performed to remove poorly segmented images, false positive registration due to tissue movement or loss and non-cellular artifacts created by over-segmentation, resulting in a removal of 5.8% additional patients. Cell level and subcellular level marker expressions were quantified using image analysis algorithms and compared between serial sections. Associations between marker expressions and histological subtypes were investigated in European male smokers. Multivariate analysis was performed using logistic regression Cox proportional hazard models on over 350 quantitated features of marker expression. All models were controlled for age and smoking status.

RESULTS & DISCUSSION

High variability readily discernible among clinically and histologically similar tumors (columns 1&2).

Sample analysis workflow

A major goal of this study was to evaluate multiplexing on the MultiOmyx® platform for biomarker profiling in NSCLC. To that end, a majority (> 2/3rd) of the measurement features evaluated showed better correlation from section variability compared to the expected patient-to-patient variability. High inter-section variance that was observed in some cases was attributed to the limited availability of one tissue section between 8.6% additional patients. Cell level and subcellular level marker expressions were quantified using image analysis algorithms and compared between serial sections. Associations between marker expressions and histological subtypes were investigated in European male smokers. Multivariate analysis was performed using logistic regression Cox proportional hazard models on over 350 quantitated features of marker expression. All models were controlled for age and smoking status.

REFERENCES

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Table 1: Protein Targets

Table 2: Clinical Characteristics

Table 3: EGFR and cMET expression

CONCLUSIONS

• The study demonstrates the capabilities of multiplexing technology (MultiOmyx® for assessment of limited lung samples, encompassing topographic expression features and the ability to observe relationships between markers through in situ profiling in individual cells.

• Additionally, by evaluating markers on exactly the same sample set (same section), a direct comparison of their relative significance in predicting course of disease is now feasible.