there are high levels of agreement amongst related cancers.

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**Optimized NGS panel and data analysis enable high sensitivity of detecting colon and lung cancer associated mutations from plasma**

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Tracking tumor-associated mutation in plasma can serve as biomarkers to facilitate cancer diagnosis and provide guidance for target therapy. The intrinsic low abundance and short fragments of circulating cell-free tumor DNA (cfDNA) make the mutation detection from plasma a challenging task. Next Generation Sequencing (NGS) allows high throughput mutation analysis of cancer genes by massively parallel sequencing. Ion AmpliSeq panel is able to construct the library with as little as 10ng DNA, suitable for the limited amount from cfDNA. However, the commercial AmpliSeq panel together with the TVC (Ion Torrent variant caller software) analysis was mainly designed for mutation detection from FFPE samples. The sensitivity is not good enough for cfDNA mutation detection based on our assessment.

Here we have established a strategy to detect somatic mutations of colon and lung cancer with plasma based on AmpliSeq Ion Torrent System. Firstly, a colon and lung panel was designed for small amplicons (less than 140bp), so as to better capture small cfDNA fragments as effective templates in multiplex PCR. The amplification test shows that our panel produced amplicons with a good uniformity when applied to cfDNA. Secondly, background noise analysis has shown the noise unevenly distributed along the genome and error hotspots repeatedly showed up among different samples. Based on the features of background noise, we have developed a new algorithm to distinguish low level mutations from sequencing errors, which makes use of sequencing data from healthy individuals as reference to estimate the sequencing error profile. A reliability of the mutant allele at each position was evaluated by statistical test of background noise versus signal. To evaluate the detection limit of our approach, a cell line mix which contains several mutations at different level of mutations was examined. Digital droplet PCR (ddPCR) assay was used to confirm the allele frequency of each mutation. This analysis demonstrates that our approach is able to call mutations at a fractional abundance of 0.15-0.3%, depending on the genomic positions. The allele frequency from sequencing result correlates well with ddPCR result ($R^2=0.90$). Moreover, the analytical specificity is higher than 99% with such detection limit.

To test the clinical performance of our method, cfDNA from non-small cell lung cancer (NSCLC) patients was used. With 10ng cfDNA, this method is able to call mutation as low as 0.5%, the lowest clinical sample tested, while with the commercial kit and variant analysis software, it only calls at 5%. The detection limit of our approach for clinical cfDNA sample will be determined with more tests.

Ion Torrent PGM has been widely used in clinical environment, because of its cost and time effective way compatible with clinical practice. In this proof-of-concept study, our strategy comprised of an optimized panel suitable for cfDNA and a novel variant calling algorithm significantly improves the detection limit of the PGM platform, making it possible to detect low abundant mutations from plasma. We envision this approach could have utility for patient stratification, monitoring response to therapies and detection of relapse.

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**Constitution of ALK fusion variants in East Asian lung adenocarcinoma**

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**Introduction:** The purpose of the study is to investigate the constitution of anaplastic lymphoma kinase (ALK) fusion variants in East Asian lung adenocarcinoma (LUAC) patients and to provide instructional data for the variants that should be included in the companion diagnostic assay to screen for ALK positive patients.

**Methods:** From June 2014 to August 2015, 158 ALK fusion positive formalin-fixed, paraffin-embedded (FFPE) samples were identified, including 3 patient-derived xenograft (PDX) tissues and 155 resected or fine needle aspiration (FNA) tissues, all derived from Korean or Chinese patients. Either purified RNA or crude tissue lysate from the 158 FFPE samples were analyzed by NanoString nCounter Elements assay with a multiplexed probe mix in a single tube targeting ALK fusion transcripts including 29 known variants.

**Results:** Of the total 158 ALK fusion positive samples, 133 samples harbored echinoderm microtubule-associated protein-like 4 ALK (EML4-ALK) fusion, 4 samples
The hyperglycolytic, hypermetabolic activity of cancer cells contributes to a ‘stressful’ local tumor environment in which cells compete for limited nutrients while being bathed in excessive metabolic waste, including high levels of lactic acid. Cells within a tumor undergo a period of environmental stress that is most pronounced when the growing tumor cell mass exceeds its vasculature, further limiting nutrients and increasing the accumulation of waste. This can be viewed as a selection step in which only those cells, both cancer and stroma, that are best adapted to survive the stress maintain the ability to proliferate. The aim of this study was to characterize the molecular basis for the survival of non-small cell lung cancer (NSCLC) cells when exposed to environmental stress. To accomplish this objective we used an in vitro culture system designed to recapitulate the environmental stressed conditions of depleted nutrients and accumulated waste experienced by cells within tumors. In examining a panel of human NSCLC cell lines, we unexpectedly found that the status of RAS was a more important determinant for surviving environmental stress than the status of p53. Lines with activating RAS mutations (KRAS or NRAS) were better poised to survive severe environmental stress, independent of p53 status. In detailed molecular cell biological studies comparing a NSCLC cell line wild type for p53 with an activating KRAS mutation to a NSCLC cell line mutant for p53 without an activating RAS mutation, we found that survival correlated with the level of autophagy in the unstressed cells. Cells that survived stress had low basal levels of autophagy that increased upon stress, whereas those that did not survive stress had elevated autophagy in the absence of stress that was increased further with the stress. Basal activation of AKT (also known as Protein kinase B) was higher in unstressed KRAS cells than in p53 mutant cells. Active AKT inhibits autophagy, thereby likely explaining the lower basal level of autophagy in KRAS mutant cells. In quantitative fluorescence microscopy studies of living cells, we demonstrated that p53 mutant NSCLC cells, in addition to having elevated autophagy, have more acidic and motile lysosomes, phenotypes correlated with increased lysosomal activity. This basal ‘lysosomal activation’ is likely linked to the elevation of basal autophagy. Based on these studies we conclude that the ability of cells to maintain low autophagy in the absence of stress and to activate autophagy in response to stress are important for enhanced survival when cells are challenged with environmental stress. Activated RAS, via regulation of nutrient absorption pathways, might make cells less dependent on stress-activated mechanisms, such as autophagy, to fulfill nutrient needs. Furthermore, loss of p53 in the absence of KRAS activation results in an increased basal autophagy, which when further increased by environmental stress, results in decreased cell survival. These data suggest that the role of autophagy in cancer cell survival in the context of environmental stress is linked to specific oncogenic mutations. This finding has significant implications for targeting autophagy in the treatment of lung cancer.

Mutation status matters: RAS, p53, and targeting autophagy as a potential strategy in NSCLC

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The hyperglycolytic, hypermetabolic activity of cancer cells contributes to a ‘stressful’ local tumor environment in which cells compete for limited nutrients while being bathed in excessive metabolic waste, including high levels of lactic acid. Cells within a tumor undergo a period of environmental stress that is most pronounced when the growing tumor cell mass exceeds its vasculature, further limiting nutrients and increasing the accumulation of waste. This can be viewed as a selection step in which only those cells, both cancer and stroma, that are best adapted to survive the stress maintain the ability to proliferate. The aim of this study was to characterize the molecular basis for the survival of non-small cell lung cancer (NSCLC) cells when exposed to environmental stress. To accomplish this objective we used an in vitro culture system designed to recapitulate the environmental stressed conditions of depleted nutrients and accumulated waste experienced by cells within tumors. In examining a panel of

Unique roles for Akt isoforms in lung cancer

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Lung cancer is the leading cause of cancer-related mortalities worldwide and 5 year survival rates are typically <20%. As traditional therapies are largely ineffective