Genetic Alteration Allowing Lung Cancers to Escape the Immune System May Occur Late in Tumor Evolution

PHILADELPHIA — A specific genetic alteration that could allow cancer cells to escape the immune system was detected in 40 percent of non–small cell lung cancers (NSCLCs) analyzed, according to data presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, held Oct. 26-30. Data suggest that the alteration occurs late in tumor evolution.

This study is being simultaneously published in *Cell*.

"One hallmark of cancer is the ability of cancer cells to evade destruction by the immune system," said Rachel Rosenthal, a graduate student in the laboratory of Charles Swanton, PhD, at the UCL Cancer Institute at University College London, United Kingdom. "Together with Nicholas McGranahan, PhD, we developed a method to analyze whether we observed one potential mechanism of immune evasion—loss of heterozygosity (LOH) at the human leukocyte antigen (HLA) locus—in lung cancers and, if we found it to occur, to investigate its frequency and how it might impact tumor evolution."

Rosenthal explained that the presence of HLA class I molecules on the surface of cancer cells is essential for cancer cell recognition and destruction by immune cells called CD8-positive T cells, and that most cells in a human body contain two sets of genes encoding the HLA class I molecules, one set inherited from the mother and one from the father. Sometimes, genetic alterations can occur that result in loss of one set of genes; when this event, which is termed LOH, occurs at the HLA locus, it has the potential to facilitate immune evasion, she said.

"We saw that HLA LOH was a highly frequent event, occurring late in lung tumor evolution and under strong selective pressure," added Rosenthal. "These data have implications for our understanding of how the tumor may evade the immune system and for the development of novel neoantigen-targeting immunotherapies."

The researchers developed a computational tool called LOHHLA to analyze next-generation sequencing data from lung cancer samples and determine the number of HLA alleles present in the samples.

According to Rosenthal, because HLA genes are some of the most diverse in the human genome, with thousands of versions (alleles) of some of the genes, very few HLA sequencing reads

successfully match the human reference genome. This means it is not possible to identify heterozygous positions, which are required for LOH analysis, she said. LOHHLA gets around the problem of using the human reference genome by leveraging a patient's known HLA alleles to detect LOH.

The researchers used LOHHLA to analyze next-generation sequencing data from tumor samples obtained prior to treatment from 90 patients with NSCLC who were enrolled in the tracking cancer evolution through therapy (Rx) (TRACERx) study. HLA LOH was detected in 40 percent of patients. A similar frequency of HLA LOH was observed following analysis of The Cancer Genome Atlas next-generation sequencing data from 692 treatment-naïve patients with NSCLC and previously published next-generation sequencing data from 37 paired primary NSCLC/brain metastasis samples.

Further analysis showed that HLA LOH was associated with a high subclonal neoantigen burden, APOBEC-mediated mutagenesis, upregulation of cytolytic activity, and PD-L1 positivity, which Rosenthal said highlights that the immune system is actively sculpting the tumor and suggests that HLA LOH is a response to the selection pressure applied via immune activity.

She also explained that the subclonal frequencies of HLA LOH, their enrichment in metastatic sites, and occurrence as parallel events suggest that HLA LOH is an immune escape mechanism, selected later in NSCLC tumor evolution.

According to Rosenthal, the main limitation of the study is that currently only tumors from non–small cell lung cancer patients have been considered.

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Rosenthal consults for Achilles Therapeutics.

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Presentation Session: SPR02 – Spotlight on Proffered Papers Session 2: Immunogenomics and Response to Immunotherapy; Friday, Oct. 27; 6:30-7:15 p.m. ET; Terrace Ballroom, 400 Level

Title: Allele specific HLA loss and immune escape in lung cancer evolution

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Cancer cells adopt a variety of mechanisms to evade the immune system and avoid T-cell recognition. Disruption of human leukocyte antigen (HLA), which may lead to reduced neoantigen presentation, has been proposed as an immune escape strategy in many cancers, including lung cancer. Mutations in HLA class I genes are infrequent in early stage non-small cell lung cancers (NSCLC), suggesting alternative mechanisms of HLA disruption may be common. To date, the polymorphic nature of the locus has precluded copy number analysis and exploration of HLA loss. To investigate the prevalence and importance of HLA disruption, we present LOHHLA (Loss Of Heterozygosity in Human Leukocyte Antigen), a computational tool to determine HLA allele-specific copy number from sequencing data. Building upon previous work imputing HLA haplotypes from sequencing data (Shukla, 2015; Szolek, 2014) and utilizing previously published datasets (Jamal-Hanjani, 2017; Brastianos, 2015), we endeavored to address the prevalence and timing of HLA LOH in lung cancer and its impact on tumor evolution and neoantigen presentation. Using LOHHLA, we find HLA LOH occurs in 40% of NSCLCs and is associated with a high subclonal neoantigen burden, APOBEC mediated-mutagenesis, upregulation of cytolytic activity and PD-L1 positivity. The focal nature of these alterations, their subclonal frequencies, enrichment in metastatic sites, and occurrence as parallel events suggest that HLA LOH is an immune escape mechanism, selected later in NSCLC tumor evolution. Characterizing HLA LOH with LOHHLA refines neoantigen prediction and may have implications for immunotherapeutic approaches targeting neoantigens.

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