Università degli Studi di Cagliari
DIPARTIMENTO DI SCIENZE BIOMEDICHE

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Al Magnifico Rettore
Università degli Studi di Cagliari

Il sottoscritto Dott. Fabio Marongiu, nato a Cagliari il 20/09/1979, Ricercatore a tempo determinato B, settore sceintifico disciplinare MED/04, Patologia Generale, in servizio presso il Dipartimento di Scienze Biomediche dell'Università di Cagliari,

CHIEDE

ai sensi dell'art. 8 della Legge n. 349 del 18.03.1958, al fine di poter proseguire lo studio precedentemente iniziato in collaborazione con l'Università del Colorado, di poter usufruire di un periodo di congedo per motivi di ricerca, della durata di sei mesi da effettuarsi nel periodo tra fine Maggio e fine Novembre 2020, compatibilmente con gli impegni didattici e previa delibera favorevole del Consiglio di Dipartimento.

DICHIARA

di non percepire assegni, borse o premi in misura corrispondente al trattamento economico in godimento nella qualifica di ricercatore universitario e che l'ammontare degli importi eventualmente percepiti a titolo di borsa di studio per soggiorni all'estero di Professori e Ricercatori Universitari sarà un rimborso delle sole spese di viaggio e alloggio.

che in ottemperanza a quanto previsto nell'art. 4 co. 78 della L. 183/2011, il predetto congedo non viene fruito oltre il compimento del trentacinquesimo anno di anzianità di servizio.

ALLEGA

copia del programma di ricerca da svolgere nel suddetto periodo, presso il *Biochemistry and Molecular Genetics Department, University of Colorado, Anschutz Medical Campus*, in collaborazione col gruppo di ricerca del Prof. James DeGregori.

In fede
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Abstract: *The role of tissue microenvironment in the emergence and progression of Lung Adenocarcinoma*

Background: The incidence of cancer is increased with age. To date, this has been primarily ascribed to accumulation of oncogenic mutations during our lifespan. However, exposure to insults in a specific tissue (e.g. cigarette smoke in the lung) increases the risk for cancer. There is now a general consensus that smoking results in a state of chronic inflammation in the lung. Prof. James DeGregori has proposed and substantiated a model, Adaptive Oncogenesis, whereby changes to tissue environments can promote cancer initiation by selecting for cells with oncogenic mutations, because in the altered tissue context, particular oncogenic mutations can become adaptive (1-3). Thus, although smoking will contribute to lung cancers by increasing the frequency of oncogenic mutations, the impact of smoking-induced genomic alterations on cancer development will be highly limited by the state of the lung adaptive landscape. In other words, providing oncogenic mutations would not suffice in the absence of tissue landscape changes, and landscape changes are critical for lung cancer causation by increasing selection for oncogenic mutations adaptive in this new microenvironment.
Aim: The aim of this research project is to study how the inflammatory state of the lung microenvironment can influence oncogenic selection. In particular, two approaches will be used:
1) Using a CRISPR-Cas9 based adenoviral delivery system (4), we will activate the expression of the chromosomally-encoded EML4-ALK fusion protein in a small percentage of lung cells of young mice, aged mice, and co-housed young and aged mice. This will allow to establish the role of the gut and lung microbiota (which will be shared in co-housed animals vs. young or aged controls) in the adaptive oncogenesis induced in the lung. These experiments will help reveal the role for aging and the age-associated microbiota, in promoting the selection for EML4-ALK fusions: we hypothesize that oncogene-initiated expansions will be suppressed in young tissues but promoted in the old lung microenvironment; we also hypothesize that the microbiota of co-housed aged animals, will be rejuvenated and protective against the selection of oncogene-initiated clones.
2) Using peripheral brushings (at both the nodule site and a remote site in a contralateral lobe) from individuals undergoing workup for CT detected lung nodules, we will evaluate epithelial oncogenic clonal expansions and distal airway progenitor function, to determine whether changes in these parameters, most importantly at the remote site, reflect a microenvironment supportive of evolution to adenocarcinoma.
Methods: We will use a novel protocol, developed by DeGregori's lab, that employs custom Illumina TruSeq capture with added unique molecular identifiers (UMIs), with alterations in the protocol to greatly (~100X) increase capture efficiency. This protocol is dubbed FERMI (Fast Extremely Rare Mutation Identification) (5). By very deep sequencing (>10^6 X) of short genomic DNA (gDNA) segments (~4 kb total), together with novel bioinformatic elimination of PCR and sequencing errors (leveraging the UMIs), we can identify mutations present in primary tissues to about 10^{-4} frequency and their clonal prevalence. The analysis will focus on a small set of oncogenes and tumor suppressor genes. By sequencing deep enough to detect rare mutations, and by comparing neutral (Tier III) and oncogenic mutation frequency, we can directly assess selection for certain mutations.
Expected results: Increased VAFs reflect clonal expansion of the cells with the identified mutation, which we propose will reflect a microenvironment conducive for such expansion. We expect somatic mutational events associated with adenocarcinomas will be more common and at higher allelic frequencies in subjects with prevalent lung cancer, even at sites remote from the cancer, and in older animals. Thus, these increases in VAFs will indicate a lung landscape conducive to oncogenic adaptation and supportive of evolution to adenocarcinoma.

References:

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