Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

For my PhD project I studied the mutations in the DNA obtained from Malignant Pleural Mesothelioma (MPM) samples. I performed bio-statistical and bioinformatics analysis on the NGS-derived data to elicit somatic mutations. Afterwards I tracked those mutations in liquid biopsies from the same patients, namely pleural fluid and plasma.
If successful this experiment will allow a quicker and more accurate diagnosis, and provide a faster way to perform follow-up tests, with the advantage of having a non-invasive procedure to obtain the needed data.

Methods

The mutations were found via exome sequencing, performed on the various samples using Illumina reagents and sequencer.
The samples we examined are from different groups, from three different hospitals. One (group PI) is composed by 12 trios, each consisting of whole blood, tumor biopsy and plasma from the same person. We did NGS experiments on both blood and tumor samples so that we could be able to distinguish germinal and somatic mutations using statistical analysis of the read count for each mutation. I employed various kind of bioinformatics tools and I filtered the results in order to get the more accurate and significant among the total mutations.
The second group (group GE) is composed by 18 pairs consisting of tumor biopsies and pleural fluid. The sequencing was performed on tumor samples only, and the results were analyzed statistically to find mutations with a higher probability of being somatic.
Another group of 5 patients (each presenting tumor biopsy, blood, pleural fluid and plasma samples) was obtained thanks to the collaboration with Dr Metintas, from Eskisehir Osmangazi University in Turkey. The same sequencing and filtering procedure were applied to this group, as for group PI.
I finally performed digital droplets PCR (ddPCR) experiments on the samples, tracking down the previously identified mutations.
In order to gain knowledge on a new mutation detection technique called SHERLOCK, which was developed at MIT in Boston, I went there to start the abroad semester. Unfortunately, a few weeks after my arrive, the COVID-19 emergency forced the laboratory I should have worked in to close. This led to my early return to Italy, and I did not have the time to actually work with SHERLOCK.

Results

ddPCR experiments confirmed most of the analyzed mutations in tumor biopsies and pleural fluids. We also tracked down the mutated tumor DNA in plasma samples, although not all of the subjects have tested positive for this kind of sample. I am currently searching for other possible somatic mutations, and we are looking forward to expand the potential of our findings to other body fluids (i.e. urine), while considering the use of yet more accurate and sensible techniques to track circulating tumor DNA in liquid biopsies.