Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

For my PhD project I studied the DNA sequence of Malignant Pleural Mesothelioma (MPM) samples with the purpose of finding rare somatic mutations that characterize this kind of tumor. Once I defined a set of mutations in our samples, I tracked mutated DNA in body fluids, i.e. pleural fluid and plasma.
If successful this experiment will open a new way to get a quicker and accurate diagnosis, and provide a good way to monitor the patient's cure progression; additionally, it would be possible to perform tests with a non-invasive procedure.

Methods

The mutations are 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 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 were able to distinguish germinal and somatic mutations using statistical analysis of the read count for each mutation.
The second group 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.
I further filtered results for read quality, read count, and allele frequency; with the aim to keep only rare mutations and discard germline SNPs and sequencing errors. The selected mutations were validated via qPCR using a pair of mutation-specific primers.
I'm going to start a series of Droplet Digital PCR (ddPCR) experiments to confirm the presence of mutated DNA molecules as circulating nucleic acid in pleural fluid and possibly plasma.

Results

A good part of the mutations found are located on genes that are already present in MPM somatic mutations databases, others are new. Of those we initially identified, about seven out of ten were confirmed after validation. The ddPCR part of the experiment is starting now, after a test of this technique on some of the samples.