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WGS in patients under W&S: a preliminary analysis

Chiara Colombo, MD, Surgical Oncology, Istituto Tumori di Milano

Background

An observational approach is routinely proposed to patients with sporadic desmoid-type fibromatosis considering the unpredictable behavior of this disease (prolonged stability and spontaneous regression). Patients with progressive disease are generally treated. Molecular predictors are needed at the beginning of the disease history. The aim of this study is to identify specific genome alterations beyond the well-known β -catenin mutation in patients under wait and see approach.

Methods

Whole exome sequencing (WES) was performed on DNA isolated from fresh frozen tumor biopsies and from matched normal peripheral blood DNA. DNA was extracted with QiAmp DNA mini Kit (Qiagen) following manufacturer's instructions. Whole exome libraries were prepared in accordance with Nextera Rapid Capture Exome Enrichment protocol (Illumina) and then sequenced at 100bp in paired end mode on Illumina HiScanSQ Sequencer. After adapter removal and quality trimming, the short reads were mapped on the human reference genome hg19 with BWA software. The alignments were processed with samtools to remove PCR duplicate and with GATK in order to perform local realignment around the indel position, base quality score recalibration and insertion/deletion calling, while variation calling was performed with MuTect thus identifying all the point mutations in the sample. Analysis of CNV was performed starting from WES data with FREEC software.

Results

Up to now, 7 cases were analyzed. Two patients were excluded because the samples were insufficient. Five patients were included in this report, of which 4 cases carrying *CTNNB1* mutations (3 cases p.T41A and 1 p.S45F) and 1 case *CTNNB1* WT. An average coverage of 50X was achieved and an average of 4,2 coding non-synonymous somatic variants were detected. All samples had a stable genotype, with almost no copy number alterations and few somatic mutations in addition to *CTNNB1*. Also the *CTNNB1* WT case had a predominantly normal karyotype and carried 8 somatic mutations in coding genes. Among the somatic mutations detected in this latter patient, a heterozygous p.V92M mutation on LAMTOR2 gene was found that was predicted by three bioinformatic tools to be pathological for the protein function. LAMTOR2 is part of the Ragulator/LAMTOR complex that is involved in the activation of mTORC1 and of extracellular signal-regulated kinase. Moreover, we detected two mutations in NRP2 gene respectively in two *CTNNB1* mutated patients: a somatic p.R572Q in one case and a germline p.S579L in the other. Interestingly, both mutations were predicted to be pathological and both were located on the coagulation factor domain of NRP2, a domain known to be responsible of VEGF and semaphoring binding.

Conclusions

These findings confirmed that sporadic desmoid-type fibromatosis generally has a stable genotype, with detection of only few somatic alterations. Further sequencing analysis on tumor

biopsies and matched peripheral blood are ongoing to confirm the occurrence of NRP2. We also plan to sequence LAMTOR2 on FFPE samples of WT desmoid-type fibromatosis.