Detection of somatic mutations in Wilms tumours using gene panel sequencing

Silva C1, Carpinteiro D1, Sampaio DA1, Vieira L1

1Unidade de Tecnologia e Inovação, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa

Introduction: Wilms tumour (WT) is an embryonal kidney neoplasia in which the causative mutations are largely unknown. However, approximately one third of patients display somatic mutations in WT1, CTNNB1, TP53 and/or WTX genes, prompting the design of molecular tests to determine the mutational profile of each patient. In this work we describe a novel molecular assay based on next-generation sequencing (NGS) technology which we used to identify mutations in 36 Portuguese WT patients.

Methods: Design Studio (Illumina) was used to create a sequencing panel of 83 PCR amplicons covering 12,306 bases of exonic sequences of WT1, CTNNB1, TP53 and WTX genes. Amplicons were prepared from tumour and matched peripheral blood DNA samples (n=73) using a TruSeq Custom Amplicon kit (Illumina). Libraries were sequenced on a MiSeq instrument using paired-end 250 bp reads. Sequence reads were aligned to hg19 human genome reference sequence using MiSeq Reporter software (Illumina). Variants were annotated using publicly available databases.

Results: Data analysis of the constitutional DNA of WT patients showed the existence of 31 germline variants, including 9 variants not described in the human dbSNP database. Comparison of matched tumour samples revealed the presence of 14 putative mutations in 12 patients. The mutations included WT1 (n=3), CTNNB1 (n=4), WTX (n=5) and TP53 (n=2). In one patient, concomitant WT1 and CTNNB1 mutations were found. Comparison of results with previous Sanger sequencing data for WT1 and CTNNB1 in the same samples confirmed 5 out of 7 mutations detected by NGS in which the mutated allele frequency was above 20%.

Discussion: We conclude that gene panel sequencing is a fast and sensitive molecular assay for identification of recurrent somatic mutations in WT. However, because two thirds of patients lack known mutations, other NGS-based approaches such as exome sequencing may be fruitful to identify novel mutations in WT.