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Sequence determination of light chains causing cast nephropathy by de novo sequencing

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Objectives : In patients with light chain cast nephropathy (LCCN), monoclonal immunoglobulin free light chains (FLCs) are produced in abundance, which can be speculated that their amino acid sequences play a vital role. Immunoglobulin repertoire sequencing (5′ rapid amplification of cDNA ends sequencing, 5′ RACE-seq) has been proven to be a sensitive method to provide full-length V(D)J region (variable, diversity and joining genes) of FLCs using bone marrow samples. Considering the invasiveness and difficulty of sample acquisition, we establish here a de novo sequencing workflow for patient-derived FLCs, based on the bottom-up proteomics independent on database search.
Methods : The nucleotide sequences of light chains were determined by 5′ RACE-seq using patients-derived bone marrow samples. Purified FLCs from urine were analyzed through LC-MS/MS method after multiple enzymatic digestion. PEAKS software was then used for the de novo sequencing of peptides that were further assembled into full-length FLC sequences.
Results : The sequences of two monoclonal FLCs (κHBJ and κZLS) were detected by both methods. Our de novo sequencing workflow can provide full coverage of FLC sequences. Besides, the results showed 100% identity with these two methods, proving the reliability of de novo sequencing. These two FLCs, belonged to Vκ1 and Vκ3 subgroups respectively, possessed more hydrophobic or nonpolar amino acids compared with the corresponding germlines, which may be associated with the pathogenesis. The figure 1 showed sequences of VJ regions acquired from patients with LCCN compared to the corresponding germline sequences.
Conclusions : This study developed a reliable and non-invasive de novo sequencing workflow for determining monoclonal FLCs sequences, suggesting new thoughts for the pathogenesis of LCCN.

图片 1.png