CHARACTERIZATION NUCLEIC ACIDS UNWINDING AND EXPLORING ITS APPLICATION IN MIRNA DETECTION IN THE NANOPORE

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ABSTRACT

MicroRNAs (miRNAs) are a class of short (~14-27 nucleotides) noncoding RNAs that regulate gene expression at the post-transcriptional level. Aberrant expression of miRNAs has been found in all types of tumors. Reverse transcription real-time polymerase chain reaction (qRT-PCR) and microarrays have been developed for detecting microRNA; however, these methods need labeling and amplification, and suffer from cross-hybridization, low selectivity and lack of valid internal controls. The development of nanopore sensors for microRNA detection is a new effort. One of the superior properties of nanopore is that the ion current in a nanometer-scaled pore structure is very sensitive to the presence, location and conformation of single target molecules occupying the ion pathway.

Here we show that a nanopore sensor based on the alpha-hemolysin protein can selectively detect microRNAs at the single molecular level in the plasma samples of lung cancer patients without labeling and amplification of microRNAs. First, we uncovered a signature current pattern that can be used to electrically track the double-stranded DNA (dsDNA) unzipping process. With the signature signals, we can also distinguish the release of dsDNA without unzipping. Second, based on the electrical signatures we identified, we have designed a nanopore-based microRNA sensor for the direct and label-free detection of target microRNA in a fluctuating background, such as plasma RNA extracts from clinical samples.

This sensor can quantify picomolar levels of cancer-associated microRNAs and can distinguish single-nucleotide differences between microRNA family members. This nanopore method can be a useful tool for quantitative studies of microRNAs, which are important for non-invasive screening and the early diagnosis of diseases such as cancer.