Development of a Single-Molecule Identification Method for Hydroxymethyl Cytosine in Tumor Marker Molecules

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Keywords: DNA, RNA, Single-Molecule Detection, Tunnel-Current, Epigentics

Cancer is the most common cause of death in Japan, and it is said that one in two people will develop cancer at some point in their lifetime [1)]. Early diagnosis can reduce the burden of treatment and lower the mortality rate [2)]. Therefore, there is a demand for fast and accurate diagnostic methods. Hydroxymethyl cytidine (5-hmC: See Figure) is gaining attention as an epigenetic marker for cancer. 5-hmC can be detected before symptoms appear, enabling early detection. Conventional detection methods include mass spectrometry and conversion of modified nucleic acids (5-hmC) to different nucleobases followed by sequencing.

In this study, we focused on hydroxymethyl cytidine (5-hmC), an oxidized modification of cytosine in DNA and RNA, and employed single-molecule quantum measurement for the detection of this modified molecule (5-hmC). This method has the advantage of directly detecting and identifying molecules in an aqueous solution on a single-molecule basis. So far, we have successfully identified single molecules of nucleobases and amino acids using highly sensitive and rapid electrical measurement technology with a nano-gap electrode device [3)-6)]. For the measurement solution, we adjusted the concentration to 1uM using 5-hmC and cytidine (C), a control molecule. We conducted rapid current measurements with the nano-gap electrode. As a result, we successfully obtained current signals for each molecule. From these signals, we extracted characteristic features and attempted to identify single molecules through machine learning. As a result, we successfully created a classifier with an F-measure value of over 0.8 for distinguishing between 5-hmC and C. This technology is expected to be applied to early diagnosis and prognostic evaluation of cancer.

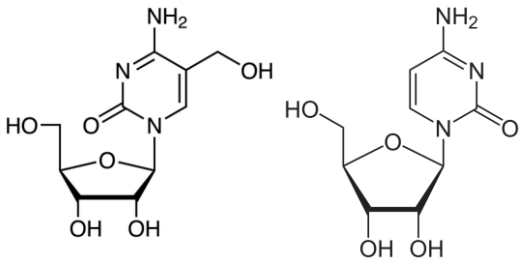

Figure:(Left) Hydroxymethyl Cytidine (5-hmC), (Right): Cytidine

Reference: 1). 国立がん研究センターがん情報サービス (https://ganjoho.jp/reg_stat/statistics/stat/summary.html) 2). 国立がん研究センター https://www.ncc.go.jp/jp/information/pr_release/2020/0317/ncc_press_release_20200317_01.pdf. 3) *Sci. Rep.* 2021, 11, 19304, 4) *Sci.Rep.*, 2019, 9, 3886, 5) *Nat. Nanotech.* 2014, 9,835-840 6) *J. Phys. Chem. C*, 2019, 123, 15867−15873