Single Living Cell Analysis Nanoplatform for High-Throughput Interrogation of Gene Mutation and Cellular Behavior

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The specific gene mutation may make the cancer cell significantly different from its non-mutant counterparts, with more aggressive behaviors, such as drug resistance[1]. The genetic heterogeneities in cancer cells pose challenges to achieving precise drug treatment in a wide-applicable manner[2]. At the single-cell level, identifying the correlation of genetic properties and corresponding cellular behaviors has become a crucial aim to precision medicine. However, traditional methods for intracellular analysis rely on cell lysis for gene extraction and amplification, showing limited capacity to provide comprehensive cues of the correlation of the genetic expression and the cellular behaviors with single-cell resolution[3].

In this work, we report a versatile single living cell analysis (SLCA) nanoplatform for high-throughput identifying the tumor gene mutant heterogeneities and in-situ tracking the behaviors (**Figure 1a-1b**). To identify mutant RNAs in single cell even with low level of gene expression, we designed a "Domino-probe" that accelerates the reaction speed by 4-fold and generates more than 10-fold amplified fluorescence signals, compared to conventional intracellular probes (**Figure 2a-2b**). A nanopore electro-delivery nanochip was developed to provide a focused electric field for safely perforating the cell membrane and instantaneously driving the Domino-probe into millions of single cells cultured on the chip (**Figure 2c**). The platform offers culturing cell array (> 10 thousand cells per chip), on-chip identifying RNA mutation, tracking cellular behaviors, and correlating the information in single living cell level (**Figure 2d**). By the system, we observed a strong correlation between the EGFR mutations and the drug resistance in lung cancer cells from clinical patient samples (**Figure 2e-2f**). We therefore demonstrated the versatility of the platform for discovering mutant subtype and drug resistance assessment in tumor cell samples, further indicated its potentials for personalizing drug treatment in lung cancer with a well-defined and tunable manner. Aiming to the emerging needs of more predictive tools for precision and personalized medicine, our SLCA nanoplatform may provide special and clinically oriented supports for drug screening and cancer therapy.

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[1] Quintanal-Villalonga, A. *et al*. 2020, Nature Reviews Clinical Oncology, 17, 360.
[2] Vasan, N. *et al*. 2019, Nature, 575, 299.
[3] Sheng, K. *et al*. 2017, Nature Methods, 14, 267.

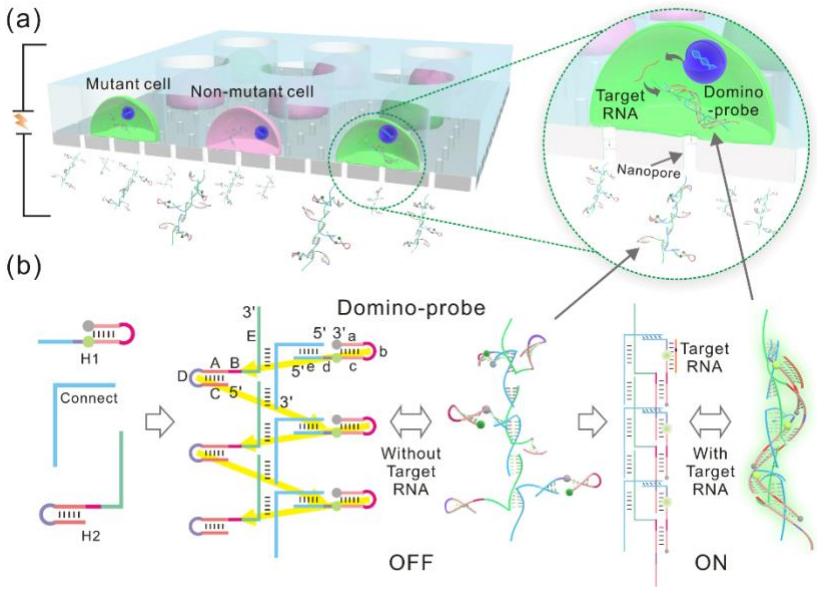

Figure 1. Principle of SLCA nanoplatform: (a) Schematic illustration of the principle of microwell array-based nanochip for detecting target RNA in the single living cells. (b) Schematic structure of the Domino-probe and its allosteric transformation based on double-helical strand displacement before (OFF) and after (ON) binding with target RNA.

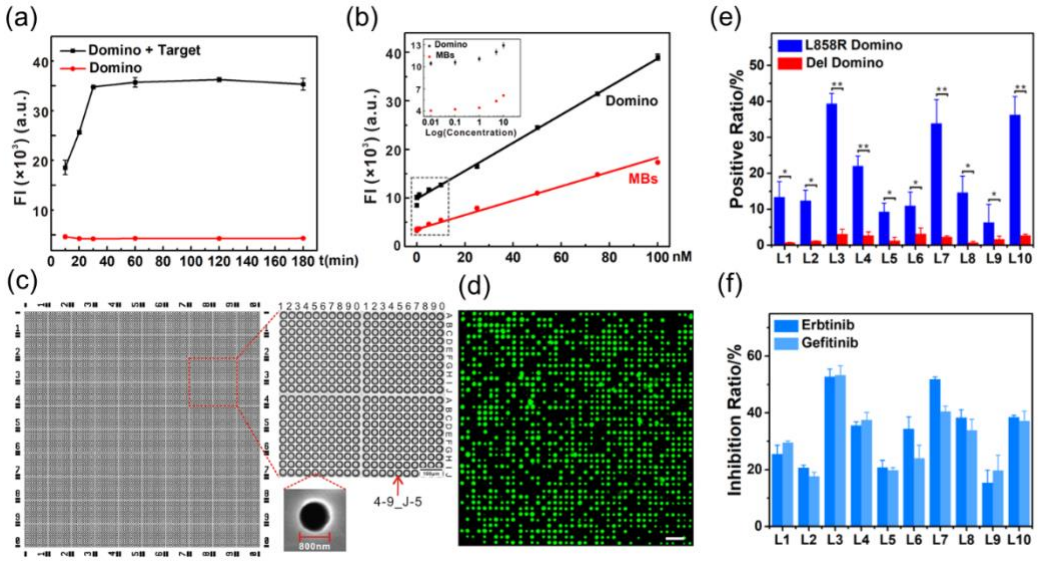

Figure 2. Functional verification of SLCA nanoplatform: (a) Response time of the Domino-probe for detecting 100 nM of target RNA. (b) Calibration curves of the Domino-probe and molecular beacons (MBs) for the detection of target RNA in vitro. (c) Layout of microwell array for patterning cells. The enlarged image shows a nanopore (800 nm) on the bottom of the microwell. (d) The patterned single-cell array on microwell array. Green fluorescence, Calcein AM. Scale bar: 100 μm. (e) Ratio of mutation-positive cells detected from primary tumor cell samples with EGFR L858R mutation. (f) The inhibition of EGFR mutation-targeted drugs to the primary tumor cells with EGFR L858R mutation.