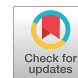
Biomaterials 156 (2018) 56–64

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A gapmer aptamer nanobiosensor for real-time monitoring of transcription and translation in single cells



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ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
23 October 2017
Accepted 21 November 2017
Available online 24 November 2017

Keywords:
Nanobiosensor
Gapmer
Aptamer
Locked nucleic acid
Gene expression

ABSTRACT

Transcription and translation are under tight spatiotemporal regulation among cells to coordinate multicellular organization. Methods that allow massively parallel detection of gene expression dynamics at the single cell level are required for elucidating the complex regulatory mechanisms. Here we present a multiplex nanobiosensor for real-time monitoring of protein and mRNA expression dynamics in live cells based on gapmer aptamers and complementary locked nucleic acid probes. Using the multiplex nanobiosensor, we quantified spatiotemporal dynamics of vascular endothelial growth factor A mRNA and protein expressions in single human endothelial cells during microvascular self-organization. Our results revealed distinct gene regulatory processes in the heterogeneous cell subpopulations.

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1. Introduction

In multicellular processes such as cellular self-organization during tissue development and regeneration, cells are subjected to spatiotemporal regulation directed by cell-cell communication and environmental stimuli for forming complex tissue architectures [1]. Gene expressions in each cell are dynamically regulated throughout various synthesis and degradation pathways [2,3]. The abundances of mRNA and protein are often poorly correlated due to the diverse regulatory mechanisms, such as transcription factors, *cis*-regulatory RNA elements, post-transcriptional modifications, RNA interference, RNA binding proteins, and ubiquitination [4,5]. Effective methods for massively parallel detection of transcription and translation dynamics at the single cell level are required for defining cell states and elucidating the multicellular organization process.

Multiplex detection of mRNA and protein expression in a single cell can be performed in fixed and isolated cell samples using combinations of proximity ligation assays, RNA-seq, digital PCR, RNA in situ hybridization, and immunostaining [6–9]. Features of cell-cell coordination and dynamic regulatory schemes in multicellular processes, however, are inherently lost by study of cells in isolation and fixation. Fluorescent protein tagging systems, such as MS2 and SunTag, are available for dynamic gene expression analysis in a single cell [10,11]. These techniques have been applied for real-time observation of translation of single mRNA molecules in live cells [12–14]. Nevertheless, fluorescent protein tagging systems require genetic modifications, which are often impractical for studying endogenous molecules. Transfection of multiple reporter constructs in primary human cells with high yield is also challenging for probing multicellular processes during tissue morphogenesis and regeneration [15].

To address the need for high-throughput single cell analysis, we developed a nanobiosensor for intracellular detection of mRNA expression in live cells and tissues [16–18]. The nanobiosensor consists of locked nucleic acid (LNA) probes and gold nanorods (GNRs). The GNR spontaneously binds to the probes to form a GNR-LNA complex and effectively quenches the fluorophores conjugated to the LNA probe. The GNR also enables endocytic delivery of LNA probes into live cells without transfection or microinjection, facilitating massively parallel detection of gene expression dynamics in multicellular communities. With a target mRNA molecule, the LNA

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https://doi.org/10.1016/j.biomaterials.2017.11.026
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probe, which is the complementary sequence, is thermodynamically displaced from the GNR, allowing the fluorophores to fluoresce. The reversible binding reaction allows dynamic gene expression analysis in live cells and tissues. The nanobiosensor has been applied for investigating injury induced response in mouse cornea [17], photothermal ablation induced heat shock response in lung tissues [16], dynamic regulation of Notch1-Dll4 signaling during microvascular self-organization [18], the formation of leader cells during collective cell migration [19], and Nrf2 mediated chemoresistance in KRAS^{G12D} mouse lung tumor [20]. Nonetheless, multiplex single cell detection of transcriptional and translational dynamics with high resolution during cellular self-organization remains a challenging task.

In this study, we report a multiplex nanobiosensor for monitoring mRNA and protein expression dynamics in live cells simultaneously by establishing a gapmer aptamer design along with complementary LNA probes and GNRs. The gapmer aptamer nanobiosensor is optimized for detecting intracellular protein expression and distribution with high specificity and stability in live cells. The multiplex nanobiosensor is applied to monitor vascular endothelial growth factor A (VEGF-A) mRNA and protein expressions during microvascular self-organization. The nanobiosensor simultaneously tracks the mRNA and protein expressions in hundreds of cells for over 20 h, resulting in more than 100,000 expression data points along with spatial and morphological information of individual cells in a single experiment. This technique enables us to monitor the dynamic VEGF protein and mRNA from the subcellular level to the population level. The results reveal diverse mRNA and protein expression patterns in the heterogeneous subpopulations of endothelial cells, suggesting distinct gene regulation mechanisms involved in the self-organization of the multicellular community. The capability of the nanobiosensor for massively parallel mRNA and protein detection in single live cells provides a versatile detection method for identifying functional cell subpopulations and studying the gene regulatory networks in multicellular processes.

2. Results

2.1. Intracellular protein detection with gapmer aptamer

An intracellular protein nanobiosensor was developed by incorporating molecular aptamers into the GNR-LNA nanobiosensor (Fig. 1a). Unlike previous aptamer biosensors [21–23], we designed the aptamer sequence with LNA monomers to enable intracellular protein detection with high specificity and stability. Three aptamer probe designs including DNA aptamer, gapmer aptamer, and alternating aptamer were synthesized (Fig. 1b and Supplementary Table 1) [24]. A fluorophore (6-FAM) was conjugated to the 5′ end of each aptamer probe. In this intracellular nanobiosensor, the fluorophore-labeled aptamer is displaced from the GNR and fluoresces only with the existence of a target protein. The binding affinities of the aptamer probes with GNR were characterized and optimized for VEGF protein detection (Supplementary Fig. 1a-b). Endocytic internalization of GNRs enabled delivery of the nanobiosensor into cells with high efficiency and minimal toxicity for massively parallel detection of single cells and high-resolution imaging (Fig. 1c-d) [25]. The gapmer aptamer with LNA modification in both ends of the sequence was quenched by the GNR effectively and had the highest binding affinity to VEGF protein among all aptamer probes. Consistently, the gapmer aptamer resulted in the highest contrast for high-resolution imaging in live human umbilical vein endothelial cells (HUVEC) (Supplementary Fig. 1c). The nanobiosensor was also capable of detecting autoregulation of VEGF and thrombin-induced VEGF expression in microvascular structures self-assembled on basement membrane matrix (Fig. 2 and Supplementary Fig. 2). The increase in VEGF expression was in good agreement with previous VEGF studies [26,27], supporting the applicability of the aptamer nanobiosensor for intracellular protein detection. The specificity of the gapmer aptamer was verified by VEGF knockdown with RNA interference (Supplementary Fig. 3). Since the gapmer aptamer design had the highest signal-to-noise ratio, it was utilized for detecting intracellular VEGF protein in this study.

2.2. Simultaneous detection of mRNA and protein in single live cells

For simultaneous detection of VEGF mRNA and protein, a complementary nucleic acid probe sequence was designed and labeled with a different fluorophore (TEX 615). The nucleic acid probe consisted of alternating LNA-DNA monomers, which were previously optimized for intracellular mRNA detection [16–18]. Both fluorophores labeled on the mRNA and protein probes were quenched due to GNR's fluorescence quenching ability. Simultaneous detection of VEGF mRNA and protein was demonstrated in HUVEC microvascular structures (Fig. 1c and Supplementary Fig. 4a). A housekeeping gene, β-actin mRNA, was also incorporated to verify the uniformity of probe delivery (Supplementary Fig. 4b). The level of β-actin expression was uniform among the cells and maintained a constant level (Supplementary Fig. 5). Unlike transfection of molecular beacons and double-stranded probes [28–30], the nanobiosensor was delivered into the cytoplasm without nuclear accumulation (Supplementary Fig. 1c). Dynamic single cell analysis of the intracellular distribution and colocalization of VEGF mRNA and protein could be performed by incorporating high-resolution imaging (Fig. 1d).

2.3. Intracellular imaging in heterogeneous subpopulations with distinct phenotypes

We demonstrated the capability of the multiplex nanobiosensor for probing microvascular self-organization over 20 h. This microvascular self-organization assay captures the cell migration, sprouting and elongation steps during microvascular development [31–33]. In agreement with previous studies [18,32], heterogeneous cell subpopulations with distinct morphologies and phenotypes, including aggregating cells, sprouting cells and elongating cells, were observed in the experiment (Fig. 3a). The aggregating cells assembled with other cells in the nodes and maintained low values of cell perimeter and area. The sprouting cells, which connected to other cells on one end, displayed steady increases in cell perimeter and area. In contrast, the elongating cells, which connected to neighboring cells on both ends, displayed rapid increases in cell perimeter and area during microvascular self-organization. Similar microvascular networks were observed with and without the nanobiosensors (Supplementary Fig. 2). This observation suggests the nanobiosensor does not significantly interfere with the microvascular self-organization process.

We incorporate high-resolution imaging to investigate the spatial distribution and colocalization of VEGF mRNA and protein in these cell subpopulations during microvascular network formation (Fig. 3b-d). Overall, the gene expression distributions in endothelial cells were non-uniform and displayed distinct signatures among the cell subpopulations. Transient “hotspots” of VEGF mRNA and protein colocalization were observed in cells. In aggregating cells, the colocalization hotspots were observed throughout the experiment. This observation was supported quantitatively by analyzing the pixel values above the threshold (Supplementary Fig. 6). The Pearson's correlation coefficient of aggregating cells maintained a

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