proceedings

Abstract

Identification of miRNA146a in Inflammatory Macrophages Using Gold Nanoparticle Oligonucleotide Sensor Constructs †

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Background: Biomarker profiling constitutes a prominent approach to human medicine and molecular sciences. Due to their high stability and unequivocal link to signaling pathways, miRNA species are of high interest. Wide applications of miRNA profiling are currently applied in body fluids and tissue lysates of a variety of physiological and pathophysiological processes. By contrast, single cell approaches are comparatively sparse. We here applied a procedure to diagnose the nature of inflammatory versus anti-inflammatory macrophages isolated from patients with auto-inflammatory, infectious and malignant diseases using gold-nano-particle-based constructs equipped with fluorescently labeled oligonucleotides. Methods: Based on previously published procedures (Elghanian and Mirkin US2010/0167290 A1), we here optimized thethe increasing salt concentration method, to derivatize citrate-stabilized gold-nanoparticles with thiol-modified oligonucleotides, the derivatization time frame could be successfully downscaled to less than 2 h. Passivation is obtained by either alkaene thiols or polyethylene glycol thiol (PEG-SH) at concentrations of 1mM. Nanoparticles of 20 nm in diameter were functionalized with the universal docking strand 5′-CCT CCT TTA CCG TGA TTG $(SS)_3$, which was hybridized to different capture and reporter strands depending on the targeted miRNA. For the intracellular detection of miRNA 46a, the following constructs were synthesized: 5′-ACT GAA TTC CAT GGG TT–Cy3 5′-CCT CCT TTA CCG TGA TTG $(SS)_3$–Au-NP, linked with the capture strand: 3′-AC TCT TGA CTT AAG GTA CCC AA G GGA GGA AAT GGC ACT AAC. Results: After hybridization in the cytoplasm of in vitro-cultured macrophages, Cy3-specific fluorescence turned positive after 10–30 min of incubation and corresponded with NFkB translocation to the cell nucleus. The amount of nanoparticles ingested by inflammatory macrophages could be quantified by confocal laser scanning microcopy and fluorescence detection by the reflection mode. Summary: The use of probe covered nanoparticles appears to be a sensitive method to identify inflammatory macrophages at a single cell level.