Available online at www.scholarsresearchlibrary.com

Extended Abstract

Journal of Computational Methods in Molecular Design, 2021 **ISSN 2231-3176**
https://www.scholarsresearchlibrary.com/journals/journal-of-computational-methods-in-molecular-design/

Gold nanoparticles: an optical biosensor for the direct RNA quantification for cancer, neurological disorders and hepatitis C virus diagnosis

Sherif M Shawky, Helmy Institute-Zewail City of Science and Technology
E-mail: sshawky@zewailcity.edu.eg

The unique physicochemical properties of gold nanoparticles (AuNPs) have been exploited to develop gold aggregating gold (GAG) approach. Quantification of HCV RNA is a cornerstone in the infection management. On the other hand, topoisomerase 1 (TOP and tyrosyl DNA phosphodiesterase 2 (TDP2) were among the transcripts of choice due to their role as genomic stability biomarkers and their implication in various cancers and neurological disorders. The existing technologies are expensive, labour intensive and time consuming, posing significant limitations to their wide scale exploitation, particularly in economically deprived populations. We have developed for the first time; cationic AuNPs to induce aggregation of citrate capped AuNPs decorated with RNA of interest specific probe (nanoprobe). Methods: TOP1, TDP2 and HCV RNA were first captured specifically using magnetic nanoparticles that were functionalized with a TOP, TDP2 and HCV specific probes in serum specimens, respectively. The captured unamplified mRNA was then directly detected and quantified using GAG assay. Solution color was developed immediately. RNA quantification was done by recording the spectral absorbance ratio of non-aggregated AuNPs to the aggregated nanoparticles (λ530/650) against a standard curve of serial diluted RNA of interest. Results: In positive samples, the AuNPs solution retained its red color, while in negative samples the color changed to blue. A linear correlation exists between the GAG assay and the qPCR for the quantification of the RNAs with detection limit of up to 10 copies per reaction. Wild-type and TDP2 deficient cell lines confirmed the assay specificity and reproducibility in distinguishing between different transcripts. Conclusion: The novel GAG assay can be utilized as an inexpensive, rapid, simple and sensitive tool for the absolute quantification of RNA from different origins and for different applications, instead of the laborious, expensive and sophisticated real-time PCR. Moreover, it could readily be adopted for full automation. An efficient healthcare system is dependent on three main pillars: patients (disease), efficient and affordable diagnosis, as well as rapid and precise clinical decisions. Molecular diagnostics and particularly, nucleic acid testing, lay a solid foundation in effective disease management and modern healthcare strategies. In addition, advances in molecular diagnostic technologies have had a great impact on establishing the rapidly evolving pharmacogenomics field, resulting in the development of the era of personalized medicine and overall improvement in the healthcare system. DNA damage plays a significant role in cellular dysfunction and death. Defects in the DNA repair pathways result in genomic instability. In replicating cells, this could lead to cellular transformation, eventually leading to cancer development. On the other hand, in nonreplicating cells such as neuronal tissue, a consequence of loss of genomic integrity is apoptosis leading to neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Topoisomerases (TOP) and tyrosyl DNA phosphodiesterases (TDP) are among the DNA repair players that play a fundamental role in regulating gene transcription, DNA replication, recombination, and repair through different mechanisms. Failure in the activities of these proteins results in protein-linked DNA breaks (PDBs), ultimately leading to neurodegenerative diseases and cancers. Patients with neurologic disorders such as intellectual disability, seizures, and ataxia have mutations in TDP2. As a result, TDP2 expression levels are affected, leading to abortive TOP2 activity and increased hypersensitivity to TOP2-induced double-stranded breaks. Moreover, TOP1 is inevitability required for proper synaptic function and regulates the levels of multiple synaptic proteins and, thereby, its dysfunction has a negative impact on the synaptic activity. In our recent publication, we have revealed that TOP1-mediated PDBs and R-loops lead to genomic instability in mice neurons, human cells, and in spinal cord tissues of patients suffering from amyotrophic lateral sclerosis and frontotemporal dementia. Furthermore, TDP1 expression was recently found to be decreased in spinocerebellar ataxia as a result of the downregulation UCHL3. Therefore, TOP1 and TDP2 transcripts level can be utilized as potential biomarkers for many neurodegenerative disorders. On the other hand, TOP1 gene expression can be used as an early biomarker for predicting the response to TOP1-targeting chemotherapeutics. A significant correlation was found to exist in various colon and breast cancer cell lines between TOP1 expression and the sensitivity to SN-38, the active metabolite of the TOP1 poison, irinotecan. In addition, TDP2 is considered to be a potential biomarker of sensitivity to anticancer drugs such as etoposide, doxorubicin, and bicalutamide. TDP2 depletion in A549 and H460 lung cancer cell lines as well as chicken DT40 cells increased the sensitivity of the cells to etoposide. Moreover, mutant-p53-dependent overexpression of TDP2 has been implicated in cellular resistance to etoposide in lung cancer cells. Furthermore, we have recently shown that TDP1 expression is increased in rhabdomyosarcoma as a result of the upregulation of UCHL3. As a result, the expression levels of TOP1 and TDP2 can be measured and utilized as a potential biomarker in various cancers to predict and monitor patients' response to different chemotherapeutics. Sensitive and precise measurement of the mRNA transcripts expression level is of pivotal importance in enhancing our understanding to the cellular dogma, leading ultimately to accurate diagnosis and, hence, allowing physicians to make more informed clinical decisions. This opens the door for the development of more personalized therapeutic approaches, maximizing patients' benefit and overcoming the side effects and drawbacks of the current conventional therapy.

Bottom Note: *This work is partly presented at EuroScicon congress on Biochemistry, Molecular Biology & Allergy* October 11 - 12, 2018 Amsterdam, Netherlands