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Angela K. Pannier
University of Nebraska - Lincoln, apannier2@unl.edu

Zain Bengali
Northwestern University

Eric A. Ariazi
Northwestern University

V. Craig Jordan
Northwestern University

Lonnie D Shea
Northwestern University, l-shea@northwestern.edu

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Substrate-Mediated Gene Delivery for Assessment of Signal Transduction Pathways in Cancer Cells

Angela K. Pannier,[1] Zain Bengali,[1] Eric A. Ariazi,[4] V. Craig Jordan,[4] and Lonnie D. Shea [1,2,3]

1 Interdepartmental Biological Sciences Program, Northwestern University, Evanston, IL
2 Chemical and Biological Engineering; Biomedical Engineering, Northwestern University, Evanston, IL
3 Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL
4 Fox Chase Cancer Center, Philadelphia, PA

Gene delivery has the potential to be used in diagnostic applications, specifically to investigate cellular signal transduction pathways responsible for disease. Analysis of multiple pathways or genes in a parallel format can be achieved using a transfected cell array, a high throughput approach to correlate gene expression with functional cell responses, based on gene delivery from a substrate that supports cell adhesion. Substrate-mediated gene delivery functions by self-assembling DNA with nonviral vectors, resulting in positively charged complexes that can interact with a biomaterial or substrate. Cells cultured on the substrate are exposed to elevated DNA concentrations within the local microenvironment, which enhances transfection. DNA complexes can be immobilized on the substrate through specific interactions introduced through complementary functional groups on the vector and surface or through nonspecific interactions. As surface properties are critical to the efficiency of the surface delivery approach, self-assembled monolayers (SAMs) of alkanethiols on gold were used to study the mechanisms of transfection by complexes nonspecifically immobilized on chemically specific substrates. Surface hydrophilicity and ionization were found to mediate both immobilization and transfection. Additionally, SAMs were used in conjugation with soft lithographic techniques to imprint substrates with specific patterns of SAMs, resulting in patterned DNA complex deposition and transfection.

Substrate-mediated gene delivery was subsequently investigated as a means to report on the activity of signal transduction pathways. The estrogen receptor (ER) pathway in ER-positive, estrogensensitive breast cancer cells was analyzed using an estrogen response element (ERE)-regulated promoter reporter system. ER expression is an early mitogenic event in breast cancer. Upon binding of its ligand, estradiol (E2), ER activates transcription of genes containing EREs. In our study, lipoplexes, formed with a plasmid containing ERE sequences upstream of a promoter directing firefly luciferase expression, along with a plasmid containing a constitutive promoter driving renilla luciferase for transfection normalization, were immobilized on serum-modified polystyrene substrates. Surface delivery of the ERE reporter plasmid system resulted in ER responses similar to bolus delivery, with E2 inducing luciferase expression 60-70 fold over vehicle or inhibitor controls. In addition, substrate-mediated delivery of the ER reporter system eliminated variations in induction responses by different complexing agents. The ability to assess ER induction by substrate-mediated delivery is being translated to a transfected cell array, to report on the activity of multiple pathways in breast cancer.