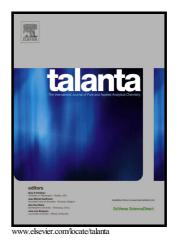
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ACCEPTED MANUSCRIPT

A Genome-Inspired, Reverse Selection Approach to Aptamer Discovery

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Abstract

Limitations of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and related methods that depend upon combinatorial oligonucleotide libraries have hindered progress in this area. Our laboratory has introduced a new approach to aptamer discovery that uses oligonucleotides with sequences drawn from the human genome to capture proteins from biological samples. Specifically, we have focused on capture of proteins in nuclear extracts from human cell lines using G-quadruplex (G4) forming genomic sequences. Previous studies identified capture of several proteins both in vitro and in live cells by the Pu28-mer sequence from the ERBB2 promoter region. Here we provide a more comprehensive study of protein capture from BT474 and MCF7 human breast cancer cells using G4-forming sequences from the CMYC, RB, VEGF and ERBB2 human oncogene promoter regions. Mass spectrometric analysis and Western blot analysis of protein capture at oligonucleotide-modified surfaces revealed capture of nucleolin by all three of the oligonucleotides in BT474 and MCF7 cells, and also of ribosomal protein L19 (RPL19) in BT474 cells. Chromatin immunoprecipitation (ChIP) analysis confirmed the interaction of nucleolin with all three promoter sequences in MCF7 cells and with RB in BT474 cells. ChIP also revealed interactions of RPL19 with CMYC in BT474 cells and of both RPL19 and ribosomal protein L14 (RPL14) with ERBB2 in BT474 cells. These results

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