New molecular insights in APE1 binding to nCaRE elements of gene promoters: identification of SIRT1 as novel target gene

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Motivation

The Apurinic/apyrimidinic endonuclease 1 (APE1) is a multifunctional protein contributing to genome stability, through its central role in the BER pathway of DNA lesions, caused by oxidizing and alkylating agents. It also plays a role in gene expression regulation, as a redox co-activator of several transcription factors and in RNA metabolism. Another interesting and yet poorly characterized function for this non-canonical DNA repair protein is associated to its ability to bind to negative calcium responsive elements (nCaRE) of some gene promoters, thus acting as a transcriptional regulator. Since nCaRE are conserved sequences located within ALU repeats, which are widespread throughout the whole human genome, many other functional nCaRE elements could exist and play a role in the transcriptional regulation of genes.

Methods

Bioinformatic analysis for the systematic retrieval of functional nCaRE sequences in the human genome was carried out filtering through biological data coming from gene expression profile of HeLa cells knocked down for APE1. Here, we developed a method that integrated classical DNA pattern matching studies with independent information on gene regulation. We used three main sources for data filtering: i) functional annotation data collected in GO; ii) gene expression data derived from the microarray profile of APE1 knock-down HeLa cells; iii) human-mouse gene sequence comparisons.

Results

As a final result, we obtained a set of genes that passed the previously mentioned filters and might be considered bona fide as candidates co-regulated through nCaRE sequences. Among these genes, whose expression is potentially regulated by APE1, attention was focused on deacetylase SIRT1 due to its involvement in cell stress, including senescence, apoptosis, tumorigenesis and its role in the deacetylation of APE1 after genotoxic stress. We showed that the human SIRT1 promoter possesses two nCaRE elements. Through a multidisciplinary approach, based on SPR, limited proteolysis, ChIP and gene reporter assays, we found that APE1 N-domain is required for the stable binding of nCaRE elements and that APE1 is part of a multi-protein complex which plays a central role in the regulatory function on SIRT1 gene especially after genotoxic stress. These findings opens new perspectives in understanding the role of nCaRE sequences on transcriptional regulation of mammalian genes.