

Supplementary figures

Figure 1: Purified WT and DM hPMC2 protein. Wild Type (WT) and the Double Mutant (DM) hPMC2 protein were purified using a Ni-NTA column. The purified proteins were then run on a SDS polyacrylamide gel, transferred onto a nitrocellulose membrane, and probed with hPMC2 antibody.

Figure 2: Active site of hPMC2. Multiple-turnover experiments, in which the enzyme concentration is lesser than the substrate, were performed with 200 nM single stranded EpRE and 20-30 nM WT hPMC2. The reaction was characterized by a burst of product formation and the amplitude of the burst was used to calculate % active protein.

Figure 3: hPMC2 exonuclease activity is required for TOT-induced QR expression in MCF10A-ER β cells. MCF10A-ER β cells were transfected with either hPMC2 miRNA that targets hPMC2 3'-UTR or hPMC2 miRNA along with plasmid expressing WT or the DM hPMC2. The cell lines were either untreated or treated with 10^{-6} M TOT for 3 hours. Proteins were extracted from cells and processed for western blot analyses of QR as described in “Materials and Methods”. Levels of QR were quantitated and normalized to GAPDH. Columns represent the fold change in QR expression levels in control or TOT-treated samples. Error bars indicate standard error of the mean of 3 independent experiments. *a*, significance ($P < 0.05$) vs. untreated cells; *b*, significance ($P < 0.05$) vs. control transfected cells with the same treatment.

Figure 4: TOT does not induce DNA strand breaks at the ERE-region of the pS2 gene.

MCF7 cells were transfected with either hPMC2 miRNA that targets hPMC2 3'-UTR or hPMC2 miRNA along with plasmid expressing WT or the DM hPMC2. Cells were either untreated or treated with 10^{-6} M TOT for 3 hours. DNA strand breaks were detected by BrdUTP labeling and ChIP assays were performed using anti-BrdU antibodies. PCR was performed with primers for the ERE region of the pS2 gene. Image shown is representative of the PCR amplification from three replicate experiments.

Figure 5: Endonuclease Activity of hPMC2. An EpRE-containing supercoiled plasmid was depurinated, treated with either APE1 or increasing concentrations of WT hPMC2 and run on an agarose gel as described in “Supplementary Methods”. APE1 converts the supercoiled plasmid into the nicked form while hPMC2 resolves it into the nicked and linear forms. Image is representative of three independent experiments.

Figure 6: A. Raloxifene does not regulate Nrf2 and hPMC2 recruitment. MCF7 cells were either untreated or treated with 10^{-6} M Raloxifene for 3 hours and processed and analyzed using ChIP assays and hPMC2/Nrf2 antibody. The EpRE containing region of the QR gene was then amplified and the image is a representative of two independent experiments. **B. hPMC2 exonuclease activity is required for enhanced Nrf2 recruitment.** MCF7 cells were transfected with hPMC2 miRNA that targets hPMC2 3'-UTR along with plasmid expressing either WT or DM hPMC2. Cells were either untreated or treated with 10^{-6} M TOT for 3 hours and processed and analyzed using ChIP assays and Nrf2 antibody. The EpRE containing region of the QR gene was then amplified and the image is a representative of two independent experiments.

Figure 7: Time course of induction of Nrf2 expression by TOT. MCF7 cells were either untreated or treated with 10^{-6} M TOT for 45 min., 90 min. or 3 hours. Proteins were extracted from cells and processed for western blot analyses of Nrf2 as described in “Materials and Methods”. Levels of Nrf2 were quantitated and normalized to Actin. Columns represent the fold change in Nrf2 expression levels in control or TOT-treated samples. Error bars indicate standard error of the mean of 3 independent experiments. *a*, significance ($P < 0.05$) vs. untreated cells.

Figure 8: Specificity of Nrf2 miRNA. Nrf2 miRNA or control MCF7 cells were treated with 10^{-6} M TOT for 3 hours. Proteins were extracted from cells and processed for western blot analyses of ER β , PARP-1, hPMC2, Topo II β and RNA pol II expression as described in “Materials and Methods”. Image shown is representative of two independent experiments.

Figure 9: hPMC2 downregulation does not affect APE1 expression levels. MCF10A cells were transfected with hPMC2 miRNA that targets hPMC2 3'-UTR. Proteins were extracted from cells and processed for western blot analyses of APE1 as described in “Materials and Methods”. Image shown is representative of two independent experiments.

Table 1: Sequence of DNA oligonucleotides cloned into expression vectors.

Supplementary Methods

Plasmids

The construction of pCMV-Flag-ER β has been described previously (Montano *et al.*, 1998). pCMV-Tag2B-hPMC2_{D247G/D386G} (DM hPMC2) was created using the Quick Change site-directed mutagenesis kit (Stratagene) using pCMV-Tag2B-hPMC2 WT (Montano *et al.*, 2000) as a template. The oligonucleotides primer pairs used for mutagenesis are listed in Supplementary Table 1. All mutations were confirmed by sequencing. pET28a-hPMC2 WT and pET28a-hPMC2 DM were constructed by NcoI digestion of pCMV-Tag2B-hPMC2 WT and pCMV-Tag2B-hPMC2 DM, respectively. The insert was blunted with Klenow and then digested with BamHI. The pET28a vector was digested with HindIII, blunted with Klenow, and then digested with BamHI. hPMC2 WT or hPMC2 DM inserts were then ligated into the pET28a vector. To make pcDNA-hPMC2 2236 and Nrf2 miRNA, the oligos encoding the miRNA sequences were annealed and cloned into the pcDNA 6.2 GW/EmGFP vector (Invitrogen) according to the manufacturer's instruction. The miRNA sequences used to construct the plasmids are listed in Supplementary Table 1 and were obtained from Sigma. In order to make the plasmid containing the EpRE of the QR gene upstream of the heterologous thymidine kinase (tk) promoter, the oligonucleotides listed in Supplementary table were annealed, gel purified and cloned into *MluI/XhoI*-digested tk-pGL3 vector. The tk-pGL3 vector was constructed by subcloning the tk-promoter containing *BamHI/BglII* insert from pTZ-tk into *BglII*-digested pGL3.

Substrate DNA preparation

The 21-nucleotide EpRE sequence 5'-TCA CAG TGA CTC AGC AGA ATC-3' was obtained from Sigma. The 5'-end of the DNA strand was labeled using [γ - 32 P] ATP and T4 polynucleotide kinase. In the case of double stranded DNA, the EpRE strand was labeled on the 5'-end and annealed to the complementary strand.

Purification of WT and DM hPMC2

BL21DE3 pLysS cells (Stratagene) were used to transform pET 28 plasmid containing either the WT or the DM hPMC2 coding sequence. Expression of WT or DM hPMC2 was induced by growing cells at 37 °C in LB medium containing chloramphenicol/kanamycin and IPTG treatment. The proteins were purified using a Ni-NTA column from Qiagen and concentrations were determined by a Bradford Assay.

Tissue Culture

Breast epithelial cells (MCF7 and MCF10A) were obtained from American Type Culture Collection and maintained as previously described (Montano and Katzenellenbogen, 1997). MCF10A-ER β cell line was generated as described previously (Sripathy *et al.*, 2008).

Transfection

MCF7 or MCF10A-ER β cells were transfected at 50 % confluency with either the respective miRNA or miRNA along with pCMV-Tag2B-hPMC2 WT or DM plasmid using FuGene HD transfection reagent. After 48 hours, cells were treated with 10⁻⁶ M TOT for 3 hours. Cells were harvested and the whole cell lysates were analyzed by ChIP or western blotting.

AP Endonuclease assay

A plasmid containing the EpRE region of the QR gene upstream of the heterologous thymidine kinase (tk) promoter was purified. This supercoiled plasmid was depurinated and the endonuclease activity was tested with APE1 and WT hPMC2 proteins based on previous work by Ando *et al.* (Ando *et al.*, 2008).