NF-kappaB regulates DNA repair-related genes and Redox metabolism in Breast Cancer Subtypes

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ABSTRACT

Breast cancer (BC) is a heterogeneous disease composed of multiple subtypes with distinct outcomes and molecular features. Human BC may be classified in three major intrinsic groups: Luminal, HER2 and triple-negative (TNBC). Oxidative stress (OS) is an important condition to genomic instability and inflammatory networks in BC. Nuclear Factor (NF)-κB signaling has risen as major player in BC pathogenesis. The correlation between NF-κB and OS has been reported in cancer, but its role in BC intrinsic groups remain to be identified. Thus, we sought to examine the role of NF-κB in BC cells focusing on understanding distinct BC subtypes. Therefore, we performed a series of global gene expression microarray analyses after NF-κB silencing in human BC cell lines MDA-MB-231 (TNBC), HCC-1954 (HER2) and MCF-7 (Luminal) compared with their scramble counterparts from each cell line. Through pathway enrichment analysis and gene ontology information, our results showed the differential expression of relevant factors involved in base excision repair, double-strand break repair, glutathione metabolism, cytochrome P450 and cyclooxygenase as result of NF-κB knockdown. Subsequently, we selected some altered genes related to DNA repair and OS for evaluation by RT-qPCR that confirmed the microarray findings. In addition, we performed biochemical analyses assessing the antioxidant capacity, lipid peroxidation profile and NO status in BC cell subtypes and correlated such parameters with the modulation of NF-κB. After NF-κB inhibition, luminal cells displayed higher antioxidant capacity at basal levels when compared to the other cells, as observed by the attenuated lipid peroxidation profile and high thiol content. HER2 cells demonstrated an intermediary oxidative profile, but with augmented thiol content and reduced lipid peroxidation. TNBC cells showed lower levels of antioxidants at basal levels, exhibiting a pro-oxidant status. As each studied cell line corresponded to one specific BC subtype, different genes were altered in response to NF-κB silencing. However, a significant number of DNA repair and OS-related genes were altered in the three studied BC models, suggesting an important relationship between these biological processes and NF-κB in BC.

RESULTS

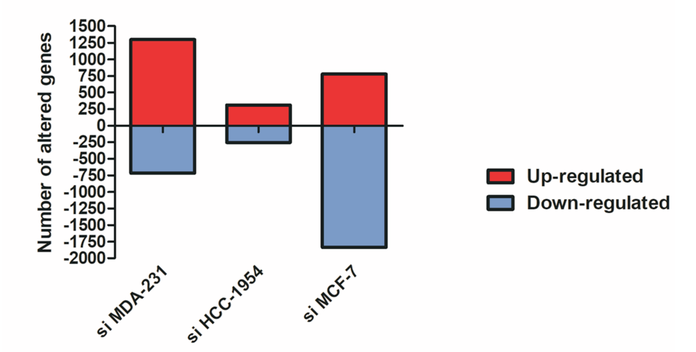

Figure 1. Differentially expressed genes identified by the chip array assay showing increased and decreased genes in breast cancer cells with silenced (si) nuclear factor-kappa B (NF-κB)/p65 compared with their scramble counterparts. Positive values (red columns) correspond to the number of up-regulated genes, and negative values (blue columns) correspond to the number of down-regulated genes.

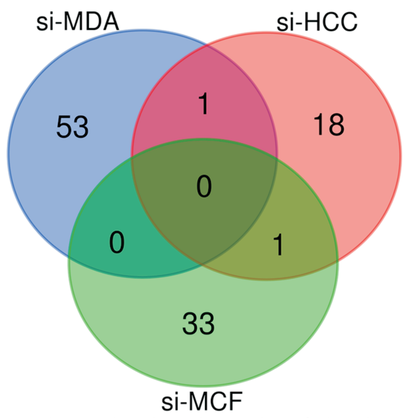

Figure 2. Venn diagram based on the number of altered genes related to redox metabolism found in each breast cancer model silenced for NF-κB/p65 compared with their scramble counterparts. Si-MDA: NF-κB/p65-silenced MDA-MB-231; si-HCC: NF-κB/p65-silenced HCC-1954; and si-MCF: NF-κB/p65-silenced MCF-7

Table 1. List of DNA repair- and oxidative stress–related genes with altered expression in NF-κB-silenced (siNFκB) breast cancer cell lines.

Fold Change	Gene Symbol	Description	GO Biological Process	GO Molecular Function
siNFκB MDA-MB-231				
11.78	*TP63*	tumor protein p63	replicative cell aging	DNA binding transcription factor activity
9.4	*PTGES*	prostaglandin E synthase	prostaglandin metabolic process; cyclooxygenase pathway; response to lipopolysaccharide; fatty acid metabolic process	glutathione binding; isomerase activity
5.01	*GPX2*	glutathione peroxidase 2 (gastrointestinal)	oxidation-reduction process; response to oxidative stress; cellular oxidant detoxification	electron carrier activity; peroxidase activity; oxidoreductase activity
2.78	*CP*	ceruloplasmin (ferroxidase)	copper ion transport; oxidation-reduction process;	ferroxidase activity; copper ion binding; chaperone binding; oxidoreductase activity; metal ion binding
2.39	*SEPW1*	selenoprotein W, 1	cell redox homeostasis	antioxidant activity; selenium binding
2.06	*HPGD*	hydroxyprostaglandin dehydrogenase 15-(NAD)	fatty acid metabolic process; prostaglandin metabolic process	NAD binding; oxidoreductase activity;
2.04	*XDH*	xanthine dehydrogenase	xanthine catabolic process; oxidation-reduction process; regulation of reactive oxygen species	iron ion binding; electron carrier activity; oxidoreductase activity, metal ion binding
1.98	*RAD51*	RAD51 recombinase	DNA repair; DNA recombinase assembly	DNA binding; recombinase activity
1.64	*POR*	P450 (cytochrome) oxidoreductase	xenobiotic metabolic process; response to nutrient	enzyme binding; hydrolase activity; electron transfer activity
−1.76	*ARG2*	arginase 2	urea cycle; arginine metabolic process; nitric oxide biosynthetic process	arginase activity; metal ion binding; hydrolase activity
−2.56	*GSTM1*	glutathione S-transferase mu 1	glutathione metabolic process; xenobiotic metabolic process	glutathione transferase activity; enzyme binding
siNFκB HCC-1954				
Fold Change	**Gene Symbol**	**Description**	**GO Biological Process**	**GO Molecular Function**
1.7	*COX11P1*	COX11 cytochrome c oxidase assembly homolog (yeast) pseudogene 1	mitochondrial electron transport, hydrogen ion transmembrane transport	cytochrome-c oxidase activity
1.64	*BRCA1*	breast cancer 1, early onset	DNA repair, double-strand break repair via homologous recombination	DNA binding, ligase activity
1.58	*FAR1*	fatty acyl CoA reductase 1	glycerophospholipid biosynthetic process; oxidation-reduction process	fatty-acyl-CoA reductase (alcohol-forming) activity; oxidoreductase activity
1.58	*RB1*	retinoblastoma 1	cell cycle checkpoint, G1/S transition of mitotic cell cycle	DNA binding transcription factor activity, enzyme binding
1.56	*HPGD*	hydroxyprostaglandin dehydrogenase 15-(NAD)	fatty acid metabolic process, prostaglandin metabolic process	NAD binding, prostaglandin E receptor activity, oxidoreductase activity
1.54	*PEX1*	peroxisomal biogenesis factor 1	peroxisome membrane biogenesis; cellular lipid metabolic process	protein binding; protein homodimerization activity
1.52	*XRCC4*	X-ray repair complementing defective repair in Chinese hamster cells 4	DNA repair, double-strand break repair	DNA binding; protein binding; ligase activity
1.51	*COX8C*	cytochrome c oxidase subunit VIIIC	mitochondrial electron transport, hydrogen ion transmembrane transport	cytochrome-c oxidase activity
−1.82	*LIPK*	lipase, family member K	lipid catabolic process; lipid metabolic process	hydrolase activity
siNFκB MCF-7				
Fold Change	**Gene Symbol**	**Description**	**GO Biological Process**	**GO Molecular Function**
1.75	*PTGS1*	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	prostaglandin biosynthetic process; fatty acid metabolic process	peroxidase activity; lipid binding; heme binding; metal ion binding; dioxygenase activity; oxidoreductase activity
1.57	*LPO*	lactoperoxidase	response to oxidative stress; hydrogen peroxide catabolic process; oxidation-reduction process	heme binding; metal ion binding; peroxidase activity; oxidoreductase activity
−1.53	*PEX1*	peroxisomal biogenesis factor 1	protein targeting to peroxisome; peroxisome organization	protein binding; ATP binding; nucleotide binding
−1.61	*SEPP1*	selenoprotein P, plasma, 1	selenium compound metabolic process; response to oxidative stress	selenium binding
−1.62	*OXR1*	oxidation resistance 1	response to oxidative stress; oxidation-reduction process	protein binding; oxidoreductase activity
−1.81	*CYCS*	cytochrome c, somatic	mitochondrial electron transport; apoptotic process	iron ion binding; electron transfer activity; heme binding
−2.19	*MDM1*	Mdm1 nuclear protein homolog (mouse)	p53 binding protein; regulation of centriole replication	protein binding
−2.76	*ATM*	ATM serine/threonine kinase; nuclear protein, ataxia-telangiectasia locus	DNA repair; telomere maintenance	transferase activity; DNA binding; protein serine/threonine kinase activity
−2.85	*ATR*	ATR serine/threonine kinase	DNA repair; cell cycle; DNA damage checkpoint	transferase activity; DNA binding; protein serine/threonine kinase activity
−3.82	*BRCA2*	breast cancer 2, early onset	double-strand break repair via homologous recombination; DNA synthesis involved in DNA repair	protease binding; histone acetyltransferase activity; protein binding; H3 histone acetyltransferase activity; H4 histone acetyltransferase activity; gamma-tubulin binding; DNA binding
−3.88	*ATRX*	alpha thalassemia/mental retardation syndrome X-linked	DNA repair; nucleosome assembly	chromatin binding; helicase activity; DNA binding; DNA helicase activity; helicase activity

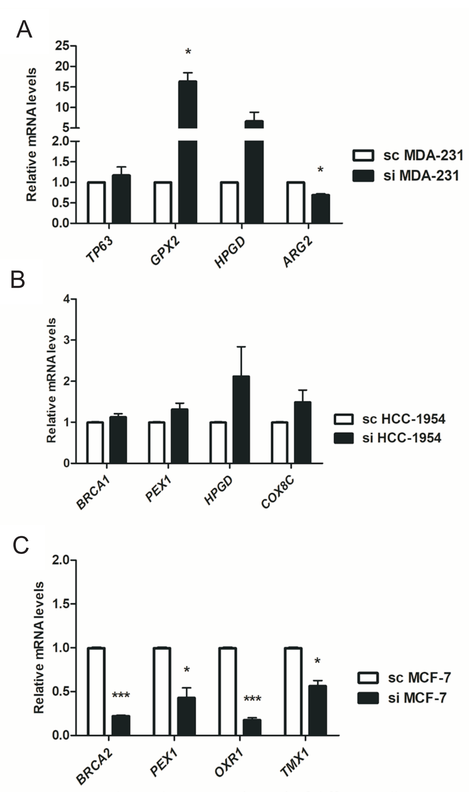

Figure 3. Relative expression by real time PCR (qPCR) of differentially expressed genes in the microarray analysis after NF-κB genetic silencing. The mRNA levels were assessed in MDA-MB-231 (A); HCC-1954 (B) and MCF-7 (C) cells, comparing the NF-κB-silenced condition (si) with the Scramble (sc) counterpart. Data are expressed as the means and standard errors of the means. *: p-value < 0.05; **: p < 0.01; ***: p < 0.001.

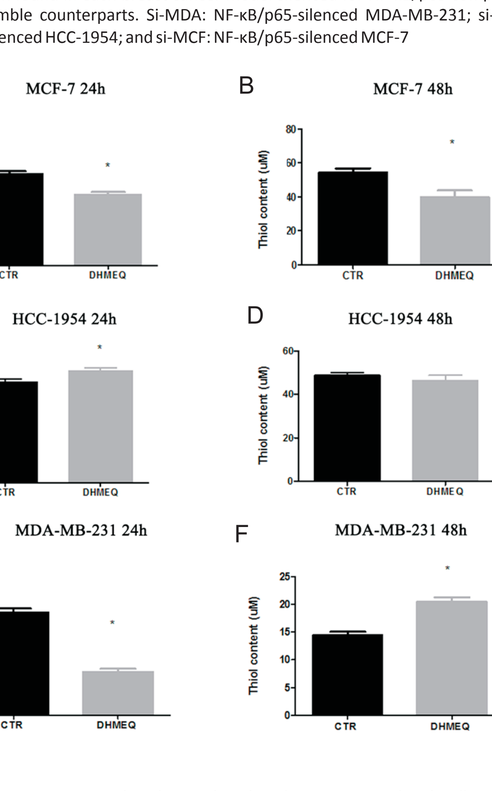

Figure 4. Thiol content. MCF-7 (A, B), HCC (C, D) and MDA-MB231 (E, F) cells treated (DHMEQ column) or not (CTR column) with the NF-κB inhibitor for 24 or 48 h. Data are expressed as the means and standard errors of the means. * indicates statistical significance (p < 0.05).

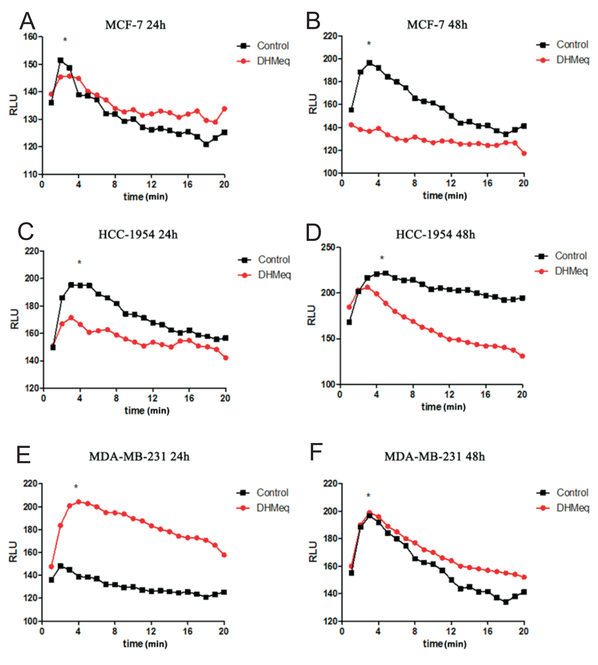

Figure 5. Lipid peroxidation profile. MCF-7 (A, B), HCC-1954 (C, D) and MDA-MB231 (E, F) cells treated or not with NF-κB inhibitor (DHMEQ) for 24 or 48 h. Data are expressed as the means. * indicates statistical significance (p < 0.05). Time (min).

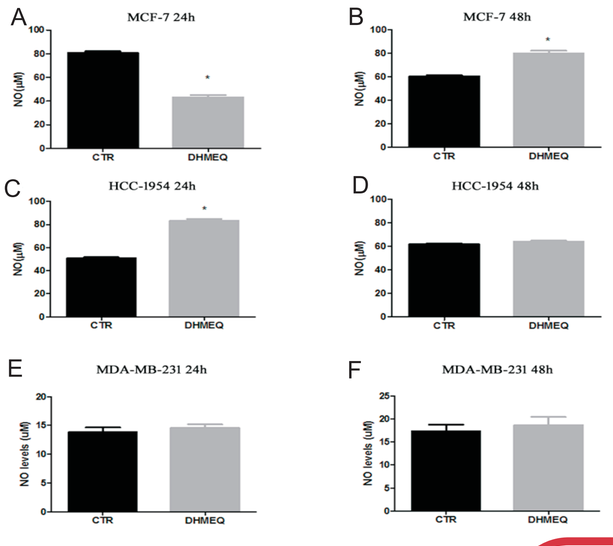

Figure 6. Nitrite as an estimate of NO levels. MCF-7 (A, B), HCC (C, D) and MDA-MB231 (E, F) cells treated (DHMEQ column) or not (CTR column) with the NF-κB inhibitor for 24 or 48 h. Data are expressed as the means and standard errors of the means. * indicates statistical significance (p < 0.05).

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA