

Genome-Wide Comparison of the Target Genes of the Reactive Oxygen Species and Non-Reactive Oxygen Species Constituents of Cold Atmospheric Plasma in Cancer Cells

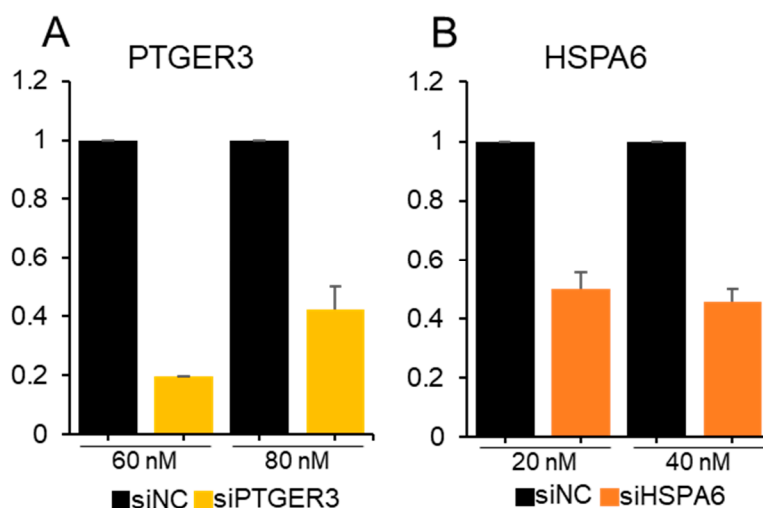


Figure S1. Induction of downregulation of PTGER3 and HSPA6 by a gene-specific siRNA in U937 cells. Cultured cells were transiently transfected with a gene-specific siRNA to downregulate PTGER3 (A) and HSPA6 (B) in U937 cells. Expression of the gene was determined by real-time RT-qPCR. Based on the downregulated level, 60 and 40 nM of siRNA were selected for PTGER3 and HSPA6, respectively. All experiments were performed in triplicate and indicated as mean \pm SE. siNC, negative control siRNA; siPTGER3, siRNA for PTGER3; siHSPA6, siRNA for HSPA6.

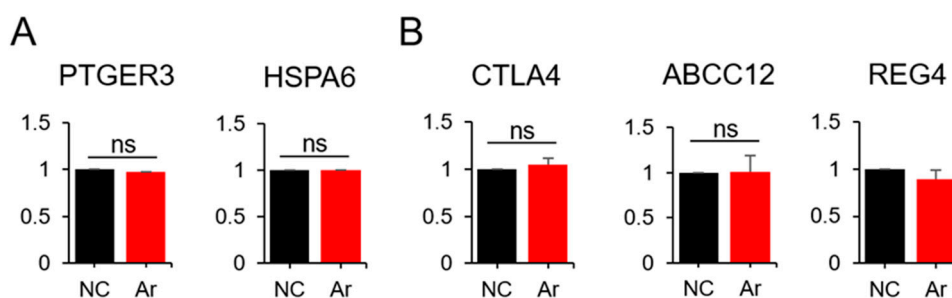


Figure S2. Effect of Ar gas on the expression of CAP- or H₂O₂-specific genes. To evaluate the effect of Ar gas alone on the expression of CAP- (A) or H₂O₂-specific genes (B), their expression was examined by RT-qPCR using RNA from the U937 cells treated with Ar gas or from non-treated cells. All assays were performed in triplicate, and the results are indicated as the mean \pm SE. ns, non-significant.

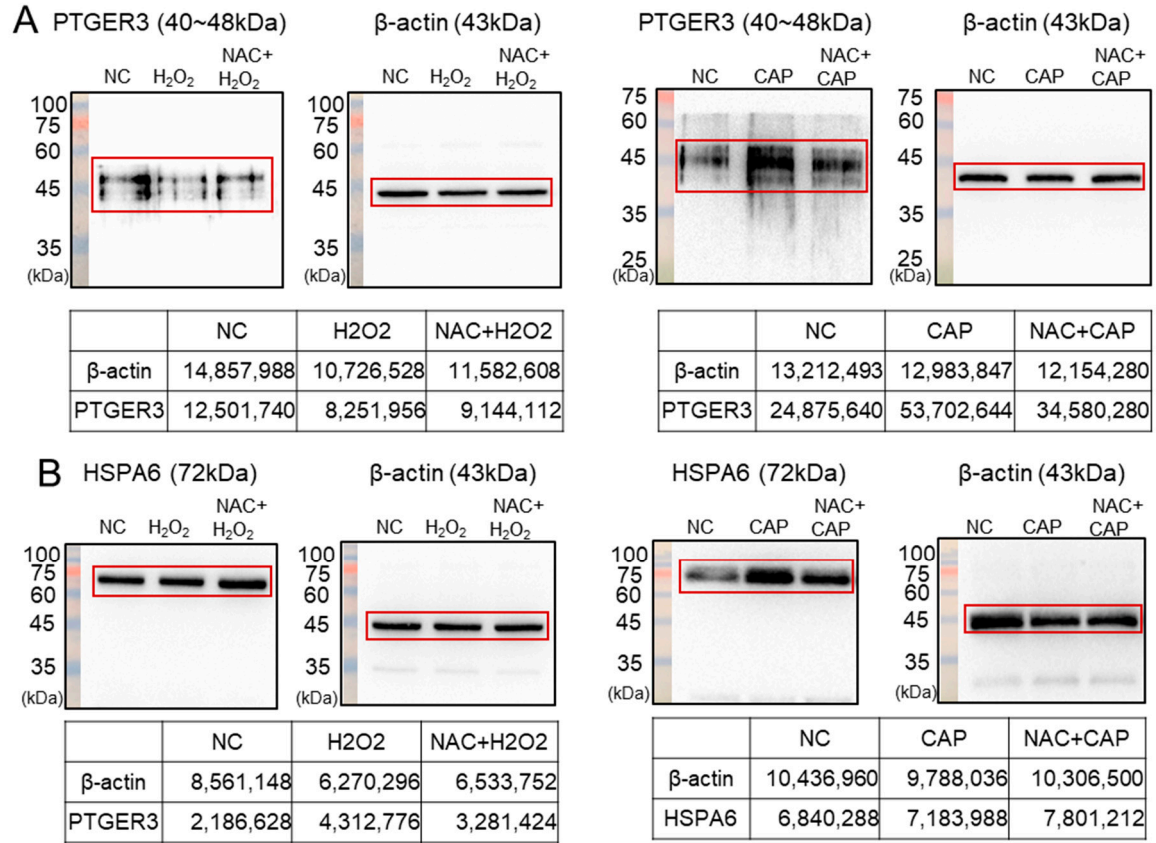


Figure S3. Uncropped blots corresponding to Western blots in the main text. Boxed areas correspond to the images in the main text. **(A)** Whole blots for Western blot analysis for Figure 5A. **(B)** Whole blots for Western blot analysis for Figure 5B.

Table S1. Information of primers for RT-qPCR and siRNAs used in this study.

Gene Symbol		Sequence (5'-3')		Supplier
qRT-PCR				
GAPDH	F:	ACATCGCTCAGACACCATG		IDT
	R:	TGTAGTTGAGGTCAATGAAGGG		
PTGER3	F:	GAGAGCAAGCGCAAGAAGTC		
	R:	CTTGGACAGGTACACGACGA		
HSPA6	F:	ACAGAGTGGCTGCCAAAAC		
	R:	CCTGTCCTCTTCGGGAATCT		
CTLA4	F:	GAGTATGCATCTCCAGGCAA		Bionics
	R:	TTCCCCATCATGTAGGTTGC		
ABCC12	F:	TGCCAAAAGATTTTCGAGTCC		
	R:	ACGCGTGTCTCTGGAATTT		
REG4	F:	TGTCATCTTGTCGTCTTCG		
	R:	CACTGTCAGCTTCTGGGTTG		
siRNA				
PTGER3	Sense:	GAAAGAUCUUCUUCGAAAAtt		Bioneer
	Antisense:	UUUCGAAGAAGGAUCUUUCtt		
HSPA6	Sense:	GUCUCCAUGUGAAAGGUUtt		
	Antisense:	AACCUUCACAUGGAAGACTt		
control		Negative control siRNA (SN-1003)		

Table S2. Genes with highly altered expression in U937 and SK-mel-147 cells after treatment with H₂O₂ and CAP.

Gene symbol	Accession No.	Description	U937		SK-mel-147		
			H ₂ O ₂ ^a				
			4 h	18 h	3 h	6 h	24 h
REG4	NM_032044	regenerating family member 4	0.429	0.371	2.990	2.791	3.520
CTLA4	NM_005214	cytotoxic T-lymphocyte associated protein 4	0.275	0.225	3.124	4.992	4.604
MEG3	NR_003531	maternally expressed 3	0.283	0.328	2.409	2.783	2.918
ABCC12	NM_033226	ATP binding cassette subfamily C member12	2.462	4.959	3.023	2.963	4.412
			CAP				
			120 s_3 h ^b	120 s_3 h ^c	20 s_3 h ^b	60 s_3 h ^b	180 s_3 h ^b
PTGER3	NM_198717	prostaglandin E receptor 3	3.317	3.411	6.685	0.380	2.136
HSPA6	NM_002155	heat shock protein family A member 6	13.642	18.379	0.439	0.458	2.209

^a The values are obtained by dividing the expression level of 3–24 h H₂O₂ treated cells by that of untreated cells. ^b The values are obtained by dividing the expression level of 20–180 s Ar-CAP treated and 3 h maintained cells by that of untreated cells. ^c The values are obtained by dividing the expression level of 120 s Ar+N₂-CAP treated and 3 h maintained cells by that of untreated cells.



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