Supplemental Materials

Molecular Biology of the Cell

Ishaq et al.

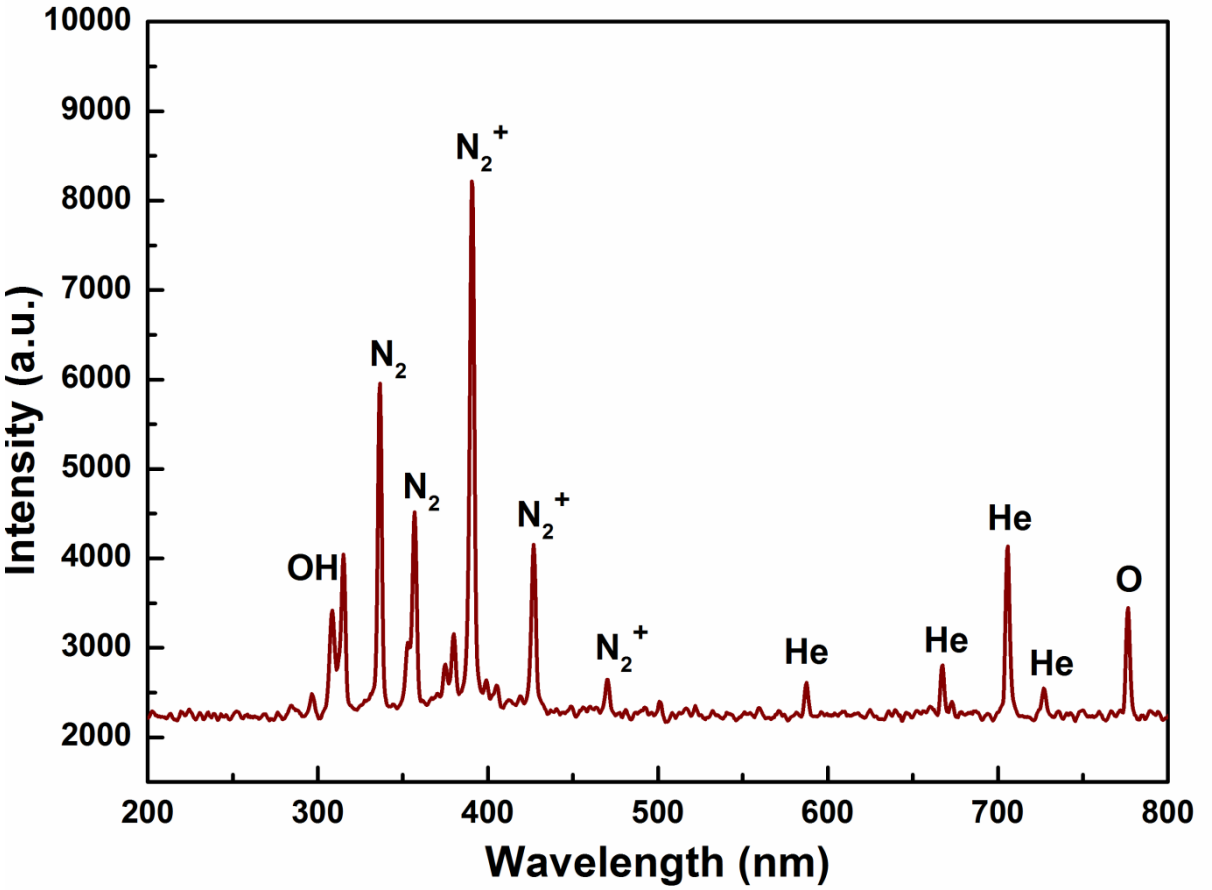
Supplementary data

Supplementary Methods

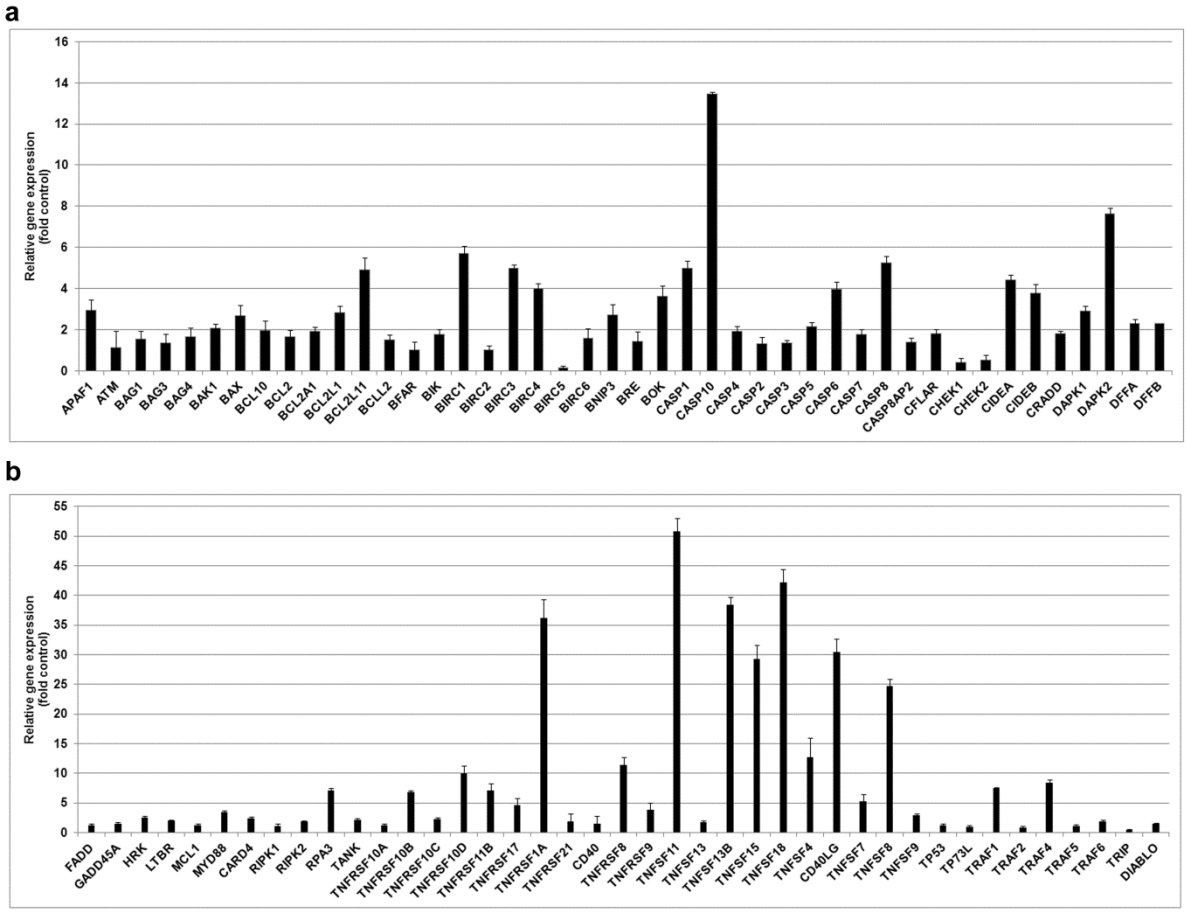
Identification of reactive species

The reactive species produced by the plasma jet were identified by collecting the emission spectra using a high-resolution emission spectroscopy (Princeton Instruments, Acton SP 2500, equipped with Pixis 256 CCD detector). The spectra (one of them is shown in Sup. Fig. 2) were collected during the cells treatment at the applied discharge voltage, Helium flow rate and RF frequency of 1.8kV, 2 L/min and 230 kHz, respectively. The wavelength calibration was performed with Hg and Ne/Ar light source prior to the collection of spectra. The spectroscope with the grating (1200 g/mm) and slit width (100 μm) were used in our experiments. Spectrum in Sup. Fig. 1 reveals that the excited species such as O, OH, He, N_2 and N_2^+ were generated in the plasma jet.

Supplementary Figures



Supplementary Fig. 1. An optical emission spectrum of the plasma jet in the wavelength range of 200-800 nm.



Supplementary Fig. 2. TNF signaling pathway family members up-regulated after AGP treatment. (a, b) Apoptosis-related intracellular differential gene expression pattern was quantified by using >90 different genes involved in apoptosis. The Mel007 cells were treated with AGP (30 sec) and relative gene expression was measured by quantitative real-time PCR after 24 hrs following the AGP treatment. Gene expression was normalized by internal genes (Actin-b, GAPDH and GUSB) and quantified as fold change compared to He gas-treated control cells. All values are mean ± s.d. of three independent experiments.

Table-1: Intracellular glutathione content of melanocytes and melanoma cells

	Melanocytes				Mel007			
	Control	AGP 5s	AGP 15s	AGP 30s	Control	AGP 5s	AGP 15s	AGP 30s
GSH (% of control)	100	93.12 ± 1.1	96.1 ± .23	92.8 ± 1.8	100	67.2 ± .23*	45 ± 1.8**	18.93 ± 2*
% Viability	91.2 ± .93	88.34 ± 2.2	90.1 ± 2.33	86.6 ± 3.1	94.2 ± 1.3	70.2 ± 1.2*	42.1 ± 1.2*	17.51± 2.4*
Total viable cells (10^5)	8.87 ± 2.0	8.67 ± 1.61	9.03 ± 1.53	8.03 ± 2.41	9.12 ± 2.02	6.8 ± 3.14*	4.36 ± .69*	2.17 ± 1.56*

Melanocytes and melanoma (Mel007) cells were treated with AGP (5, 15 and 30 sec). Total glutathione content was determined as described in materials and methods. The viable cells were counted by staining with trypan blue. The results represent the means ± s.d. of three independent experiments performed in triplicate. *$p \leq 0.01$, **$p \leq 0.001$; ANOVA.