Supplementary Materials

Identification of Redox-Sensitive Transcription Factors as Markers of Malignant Pleural Mesothelioma

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Figure S1: we performed experiments to evaluate p53 and PTEN at nuclear level. Results shown in the figure demonstrated a partially not so significative downregulation of PTEN and p53 in MPM cells towards HMC.

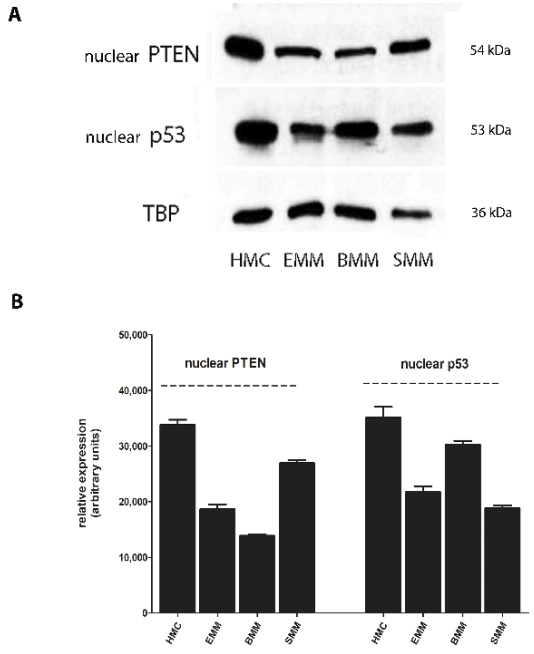

Figure S1. Nuclear expression of PTEN and p53 proteins induced by Ref-1 in MPM cells. (**A**) Western Blot of nuclear PTEN, p53 and TBP proteins in HMC, EMM, SMM and BMM cells. (**B**) Densitometric analysis ($n = 3$).

Figure S2: we performed some experiments by incubating HMC with monodispersed synthetic amorphous silica, made up of spheres (MSS), already used by our research group in the past and associated to a no toxic effect [Ghiazza M. et al. Does vitreous silica contradict the toxicity of the crystalline silica paradigm? *Chem Res Toxicol* **2010**, *23*, 620-629].
The figure shows results after 24h incubation of HMC with 80 μg/cm² MSS and 25 μg/cm² crocidolite (Croc, the higher dose used in experiments in the paper as Croc4). Nrf2, Ref-1 and FOXM1 are overexpressed only when incubated with crocidolite asbestos and not after MSS exposure:

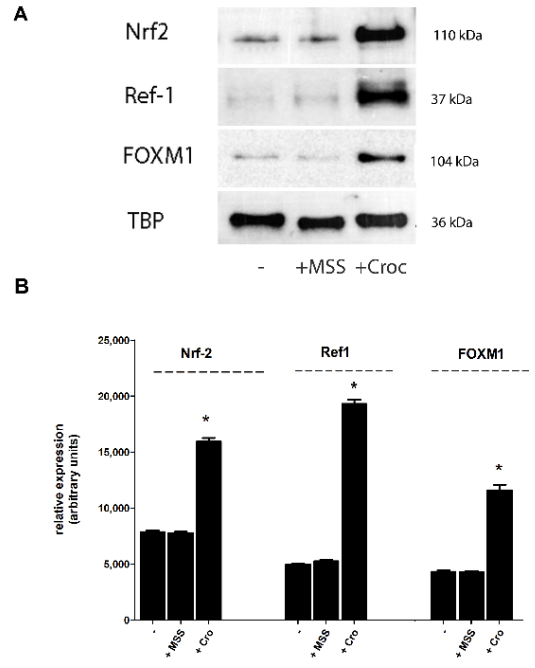

Figure S2: Increased expression of Nrf2, Ref-1 and FOXM1 after exposure. (**A**) Western Blot of nuclear extracts of Nrf2, Ref-1 and FOXM1 from HMC untreated (−) or treated (+) for 24 h with 80 μg/cm² monodispersed synthetic amorphous silica (MSS) and 25 μg/cm² crocidolite asbestos (Croc) asbestos. (**B**) Densitometric analysis of the relative expression of Nrf2 (n=3, * $p < 0.001$), Ref-1 ($n = 3$, * $p < 0.001$) and FOXM1 ($n = 3$, * $p < 0.001$) respectively.

Figure S3: we measured the basal ROS level in HMC and MPM cells (the method is described below). The results in the figure demonstrated a significantly lower level of ROS in MPM cells than in HMC, thus confirming that the overexpression of redox-sensitive transcription factors studied presumably are crucial in counteracting basal oxidative stress at the cellular level.

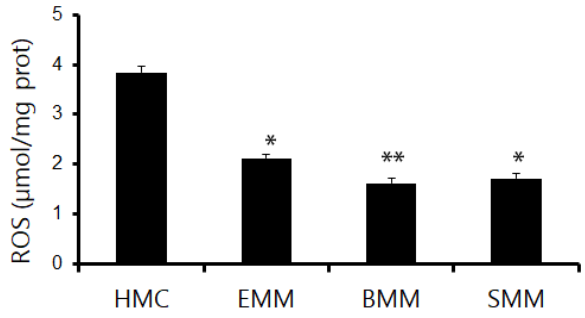

Figure S3. Intracellular ROS levels in all three histological types of MPM, epithelioid (EMM), sarcomatoid (SMM) and biphasic (BMM) forms, towards HMC. Data are presented as the means ± SD (n = 3). The results were analyzed as described in Materials and Methods: vs HMC, * $p < 0.05$; ** $p < 0.005$.

Measurement of Reactive Oxygen Species (ROS). Cells were incubated for 15 min with 10 µM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is a cell-permeable probe that is cleaved intracellularly by non-specific esterases to form DCFH, which is further oxidized by ROS to form the fluorescent compound dichlorofluorescein (DCF). After incubation with DCFH-DA the cells were washed twice with PBS to remove excess probe and DCF fluorescence was determined at excitation wavelength of 504 nm and emission wavelength of 529 nm, using a Perkin-Elmer LS-5 fluorimeter (Perkin Elmer, Shelton, CT). The fluorescence value was normalized for the protein concentration and expressed as µmol/mg cellular proteins.

Table S1: analysis data on MPM cells obtained from total 9 MPM patients, 3 for each histotype (epithelioid, biphasic, sarcomatous), of the Biological Bank of Mesothelioma (AO Nazionale di Alessandria, Italy)

MPM	MORPHOLOGY	CALRET	PANCK	D2-40	EMA	CEA	WT1	CK5	BAP1	NF2
MPM EPITELIOID										
COD 317	EPI	POS	POS	NEG	NEG	NEG	POS	NEG	POS N	POS N
COD 404	EPI	POS 50%	NEG	NEG	NEG	NEG	POS	NEG	POS N	POS N/C
COD 722	EPI	POS	POS	NEG	spor POS	spor POS	POS	NEG	NEG	POS N
MPM BIPHASIC										
COD 359	EPI	POS	POS	NEG	NEG	NEG	POS	NEG	POS N	POS N
COD 421	BIPH	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG 95%	POS N/C
COD 672	EPI	POS	POS	NEG	NEG	NEG	POS	NEG	POS N	POS N/C
MPM SARCOMATOID										
COD 353	SARC	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG 95%	POS C
COD 432	EPI	NEG	NEG	NEG	NEG	NEG	POS	NEG	POS N	POS N/C
COD 720	EPI	NEG	POS	NEG	NEG	NEG	NEG	NEG	POS N	POS N/C