

SUPPLEMENTAL MATERIAL

Materials and Methods

Determination of ROS production

Hydrogen peroxide and anion superoxide production was determined in different cell clones after incubation with 5 μ M of 2,7-dichlorodihydrofluorescein diacetate (H₂DCFDA) or with 5 μ M of MitoSOX-Red (Molecular Probes, Invitrogen, Milan, Italy) respectively, as previously described (1). H₂DCFDA fluorescence was measured at 535nm using a multilabel counter Wallac 1420 (PerkinElmer, Turku, Finland). MitoSOX-Red fluorescence was determined at 580nm with a digital imaging system using an inverted epifluorescence microscope with 63X/1.4 oil objective (Nikon Eclipse Ti-U, Nikon, Japan). Images were captured with a back-illuminated Photometrics Cascade CCD camera system (Roper Scientific, Tucson, AZ, USA) with 500ms exposure time and elaborated with Metamorph acquisition/analysis software (Universal Imaging Corp., Downingtown, PA, USA).

Supp. Table 1. α -ketoglutarate and succinate levels.

	α -KG (nmol)	Succinate (nmol)
OSC-78	7.30 \pm 1.5	3.48 \pm 0.5
OSC-83	16.65 \pm 2.1*	0.92 \pm 0.1*

Measurements of α -KG and SA were performed as described in materials and methods. Values represent mean \pm SD (n=3; * P<0.05).

Supp. Figure 1. Growth of cell lines in Rag2^{-/-}; γ c^{-/-} mice

Tumor growth curves of osteosarcoma cells lines bearing different *MTND1* m.3571insC heteroplasmy levels upon injection in mice. Data are mean \pm S.E.M. (n=3, five animals for each experiment; *P<0.05; **P<0.01).

Supp. Figure 2. Expression of HIF1 α -responding genes

Gene expression levels of HIF1 α -responding genes observed in OS-85 and OS-93 xenografts carrying m.3571insC mutation above (AT) and below threshold (BT). Absolute mRNA quantification of *GLUT-1*, *VEGF-A* and *LDHA* in tumors explanted from *nude* mice is reported. A trend of down-regulation for HIF1-responding genes is observed in tumors carrying the m.3571insC above the threshold (>83%). Statistical analysis was performed using Student's t-test and Mann-Whitney Rank Sum test for data distribution (*P<0.05). Black bars indicate the average value.

Supp. Figure 3. HIF1 α destabilization is independent from ROS production

(A) Hydrogen peroxide levels were measured using 5 μ M H₂DCFDA, as described in supplementary materials and methods. Data are mean \pm S.D. (n=3). (B) Anion superoxide production was determined using 5 μ M Mito-SOX-Red, as described in supplementary materials and methods. Images are representative of 3 different experiments. Ten images were acquired for each experiment.

Supp. Figure 4. Occurrence of mtDNA mutations in oncocytic tumors

Distribution of mtDNA mutation types and their mutant loads reported in a panel of previously characterized oncocytic tumors. More than 80% of all mtDNA mutations found in oncocytomas occurs at loads higher than the 81-83% threshold and most of these occur in genes encoding for CI subunits.

Supp. Figure 5. Authentication of 143B.TK-derived osteosarcoma cybrids via *TP53* genotyping

The *TP53* g.13055G>C mutation peculiar of 143B.TK-derived osteosarcoma cybrids is shown along with a wild-type control sequence obtained from thyroid oncocytoma-derived XTC1 cells.

Coexistence of both such *TP53* mutation and the *MTND1* m.3571insC unequivocally identifies osteosarcoma-derived cybrids.