Supplementary materials

MT2A is an early predictive biomarker of response to chemotherapy

and a potential therapeutic target in osteosarcoma

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Supplemental Table 1: Primer sequences used for qPCR on human (h) and murine (m) samples.

Name	GeneBank No	Primer sequence (5' – 3')
	NIM 002046	Forward AGCCACATCGCTCAGACAC
NGAPDH	INIVI_002046	Reverse GCCCAATACGACCAAATCC
ЬМТО А	NIM 005052	Forward TGCACCTCCTGCAAGAAA
hM12A	INM_005955	Reverse CAGCAGCTGCACTTGTCC
CADDII		Forward ACACATTGGGGGGTAGGAACA
mGAPDH	NM_008084	Reverse AACTTTGGCATTGTGGAAGG
MTO	NR 000720	Forward CATGGACCCCAACTGCTC
mM12	11111_008030	Reverse AGCAGGAGCAGCAGCTTT

Supplemental Table 2. Half maximal inhibitory concentrations (IC50) for

chemotherapy. Cell viability was assessed by the MTS test in the indicated human osteosarcoma cell lines incubated for 72 hrs in the presence of increasing doses of each indicated drug.

Coll Lino	CISP	DOXO	ETOP	IFOS	MTX
Cell Lille	(µM)	(µM)	(µM)	(µM)	(µM)
143B	2.04	0.046	0.670	12.34	0.040
CAL72	14.27	0.201	2.836	27.42	0.298
HOS	3.17	0.049	0.638	9.814	0.019
IOR/OS18	31.54	0.159	4.21	30.32	0.327
MG63	2.23	0.140	3.41	11.12	0.327
OHS4	10.813	0.098	2.751	2.431	0.006
SaOS2	3.87	0.062	2.27	17.94	0.059
U2OS	9.40	0.155	2.28	23.05	0.029















Supplemental Figure 1: Assessment of glutathione as alternative detoxification pathway. Relative glutathione (GSH) level in 143B, CAL72, HOS, IOR/OS18, MG63, OHS4, SaOS2, and U2OS cells incubated in the presence of the chemotherapeutic drugs (100 μ M cisplatinum; 1.84 μ M doxorubicin; 10 μ M etoposide; 5 mM ifosfamide or 5 mM methotrexate) or solvent, as assessed by a colorimetric assay. Results are expressed as fold change value normalized to untreated cells (mean \pm standard deviation; n = 6). An asterisk (*) indicates a statistically significant difference (p < 0.05). Box plot of distribution of relative GSH level in all cell lines incubated for 24 hrs in the presence of indicated drugs.



MT2A /GAPDH mRNA level

coefficient R² and the significance of the F test (P-value) are also given.



Supplemental Figure 3: Characterization of MT2A-silenced U2OS cell line. (A) Expression levels of MT2A in parental and stably modified U2OS cells, as assessed by RT-qPCR. GAPDH was used as internal reference gene. The relative mRNA level was calculated using the $2^{-\Delta\Delta CT}$ method and expressed as mean ± standard deviation (n = 3). An asterisk (*) indicates a statistically significant difference (p < 0.05 vs. control). (B) Representative images in parental and stably modified cells (200X magnification). (C-E) Distribution of cell shape parameters as function of the frequency of occurrence. (F) Relative DNA synthesis, as assessed by a BrdU incorporation assay. Results are expressed as fold change value normalized to parental cells (mean ± standard deviation; n = 8). (G) Relative glutathione (GSH) level in shControl- or shMT2A-modified U2OS cells incubated in the presence of the indicated drugs, as assessed by a colorimetric assay. Results are expressed as fold change value normalized to untreated cells (mean ± standard deviation; n = 4).



	CAL72		IOR/OS18		OHS4	
	Parental	shMT2A	Parental	shMT2A	Parental	shMT2A
	cells	cells	cells	cells	cells	cells
cisplatinum	4.27 μM	2.95 μM	31.5 μM	5.27 μM	10.8 µM	3.37 μM
doxorubicin	0.07 μM	0.03 μM	0.16 μM	0.07 μM	0.12 μM	0.09 µM
etoposide	0.48 μM	0.32 μM	4.21 μM	2.35 μM	2.75 μM	1.43 μM
ifosfamide	27.4 μM	8.20 μM	30.3 µM	4.51 μM	2.43 μM	0.79 μM

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	D					
_		HOS				
		Parental cells	Doxo-resistant cells			
			Control cells	shMT2A cells		
	cisplatinum	3.17 μM	2.42 μM	0.49 μM		
	doxorubicin	0.05 μM	4.39 μM	0.02 μM		
	etoposide	0.64 μM	90.5 μM	0.39 μM		
	ifosfamide	9.81 μM	10.5 μM	1.89 µM		

Supplemental Figure 4: Characterization of MT2A-silenced human cell lines. (A) Expression levels of MT2A in parental and stably modified U2OS cells, as assessed by RTqPCR. GAPDH was used as internal reference gene. The relative mRNA level was calculated using the $2^{-\Delta\Delta CT}$ method and expressed as mean ± standard deviation (n = 3). An asterisk (*) indicates a statistically significant difference (p < 0.05 vs. control). (B,D) Half maximal inhibitory concentration (IC50) values determined after 72 hrs incubation in the presence of the indicated drugs.



Supplemental Figure 5. K7M2 cells modified with shControl or shMT2 were injected IM into the thighs of SCID mice and administered with chemotherapy as described in the materials & methods section. Representative image showing Hematoxylin and Eosin (H&E) staining in K7M2 shControl- or shMT2-derived primary tumours. The scale bars represent 50 µm.



Supplemental Figure 6. K7M2 cells modified with shControl or shMT2 were injected IM into the thighs of SCID mice and administered with chemotherapy as described in the materials & methods section. Representative image showing immune-histochemical (IHC) staining for Ki67 in primary tumour FFPE sections.

Spearman correlations between the number of Ki67 positive cells, and tumour volume as expressed as mm3. The determination coefficient R^2 are also given.