Preoperative chemoradiotherapy for rectal cancer: the sensitizer role of the association between miR-375 and c-Myc

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Characteristics of the patients with rectal tumor.

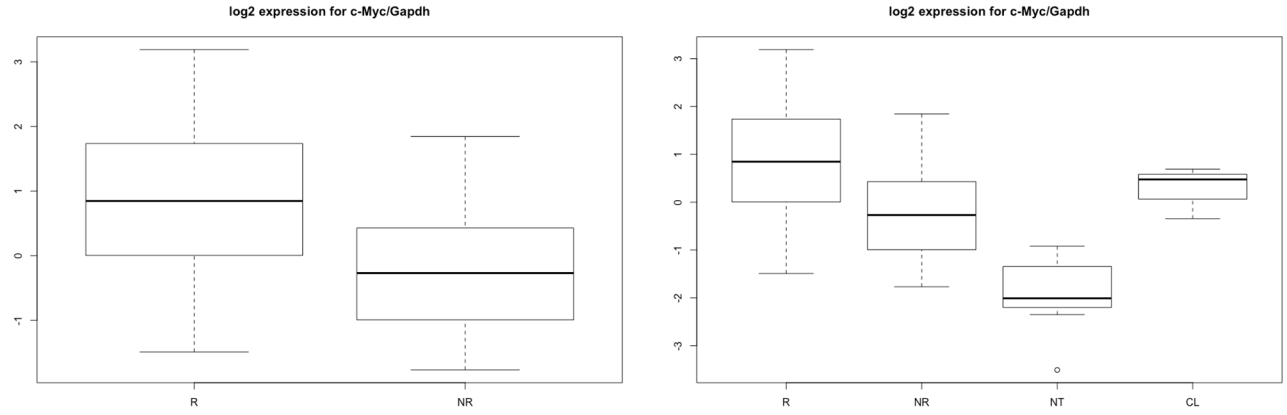
See Supplementary File 1

Supplementary Table 2: Patient characteristics stratified by response to treatment.

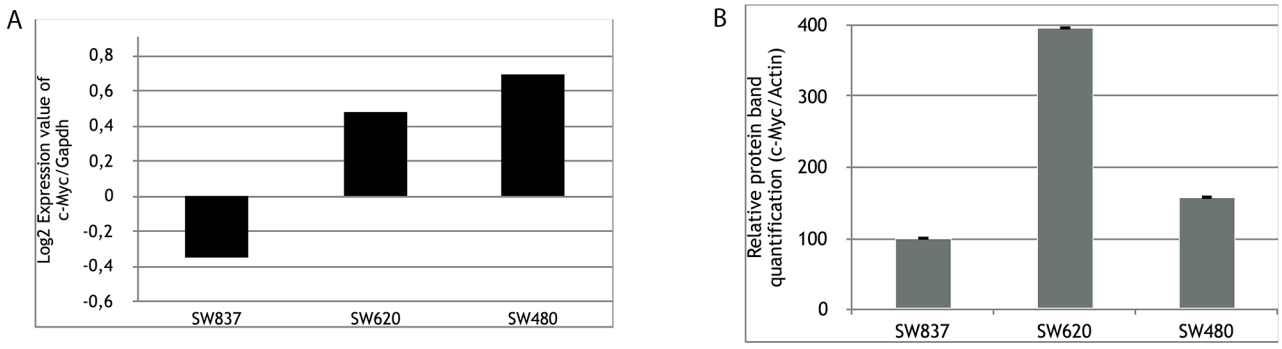
See Supplementary File 2

Supplementary Table 3: 82 miRNAs manually annotated in database as related to c-Myc gene and/or identified in the literature. 29 candidate miRNAs described in more than one database or article are highlighted in grey.

See Supplementary File 3



Supplementary Figure 1: c-Myc mRNA is a potential biomarker of response to treatment in LARC. Box plots representing expression values of gene c-Myc by real-time RT-PCR in responders (R) and non-responders (NR). Boxes represent quartiles, the median is represented by a black line within the box, and circles represent atypical values (1.5- to 3-foldthe length of the box). Significant differences in the expression of c-Myc were found among the subgroups. NT: non-tumor samples; CL: cell lines (SW480, SW620 and SW837).



Supplementary Figure 2: c-Myc mRNA and protein expression levels in SW480, SW620, and SW837. (A) TaqMan mRNA assay for c-Myc. c-Myc expression was normalized using Gapdh expression levels. SW837 cell line expressed the lowest c-Myc levels; **(B)** c-Myc protein by Western blot. Relative protein band quantification (c-Myc/Actin) was performed by optical density scanning.

Supplementary Table 4: Determination of lethal concentration (LC50) of 5-FU in S480, SW620, and SW837 cell lines

	MTT 48 h	MTT 72 h
SW480	0.95 μM	1.16 μM
SW620	0.76 μM	1.15 μM
SW837	>160 μM	80 μM

Cells were incubated with the drug for 48 h or 72 h. Viability was determined by a MTT assay to detect metabolic activity. Each sample at each concentration was run in quadruplicate and was normalized to control samples.

Supplementary Table 5: Taqman probes and primers used for the qRT-PCR analysis

	Target	Sequence	Assay ID
			Thermo Fisher Scientific
mRNA	c-Myc		Hs00153408_m1
	Gapdh		Hs02758991_g1
		miRNA database results	
miRNA	hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	477975_mir
	hsa-miR-125	UCCCUGAGACCCUAACUUGUGA	477885_mir
	hsa-miR-143-3p	UGAGAUGAAGCACUGUAGCUC	477912_mir
	hsa-miR-200c-3P	UAAUACUGCCGGGUAAUGAUGGA	478351_mir
	hsa-miR-215-5p	AUGACCUAUGAAUUGACAGAC	478516_mir
	let7c-5e	UGAGGUAGGAGGUUGUAUAGUU	478579_mir
		miRNA array results	
miRNA	miR-18a	UAAGGUGCAUCUAGUGCAGAUAG	478551_mir
	miR-30b	UGUAAACAUCCUACACUCAGCU	478007_mir
	miR-145	GUCCAGUUUUCCCAGGAAUCCCU	477916_mir
	miR-148a	UCAGUGCACUACAGAACUUUGU	477814_mir
	miR-375	UUUGUUCGUUCGGCUCGCGUGA	478074_mir
	miR-451	AAACCGUUACCAUUACUGAGUU	478107_mir
	miR-519b-3p	AAAGUGCAUCCUUUUAGAGGUU	479333_mir
	miR-650	AGGAGGCAGCGCUCUCAGGAC	479129_mir
	miR-1183	CACUGUAGGUGAUGGUGAGAGUGGGCA	477870_mir
	miR-1233	UGAGCCCUGUCCUCCCGCAG	477877_mir
	miR-1243	AACUGGAUCAAUUAUAGGAGUG	478650_mir
	let-7f	CUAUACAAUCUAUUGCCUUCCC	77801_mir
		Housekeeping	
miRNA	Snord44	CCTGGATGATGATAAGCAAATGCTGACTGAACATGAAGGTCTTAATTAGCTCTAACTGAC	CSAAYWT Bach id:w1605853025000

Supplementary Table 6: Characteristics of tumor cell lines based on online databases

	SW837 (CCL235)	SW 480 (CCL228)	SW620 (CCL 227)
Description	Rectal adenocarcinoma (stage IV)	Colorectal adenocarcinoma (Duke's type B)	Rectal adenocarcinoma (Duke's type C)
Tissue	Human rectal	Human colon	Human colon but from a lymphatic metastasis
Morphology	Adhesive epithelial cell	Adhesive epithelial cell	Adhesive epithelial cell
c-Myc mutation	p.V92I; G->A*º^ Substitution - Missense	No*º	No*º
p53 mutation	p.R248W; G->A* p.R248W; C->Tº Substitution - Missense	p.R273H; c.818 G>Aº p.P309S; c.925 C>Tº Substitution - Missense	p.R273H; c.818 G>Aº p.P309S; c.925 C>Tº Substitution - Missense
k-Ras mutation	p.G12C; C-> A* p.G12C; G->Tº Substitution - Missense	p.G12V; C->A*º Substitution - Missense	p.G12V; C->A*º Substitution - Missense
Apc mutation	p.R213; C->T*^ p.R1450; C->T*º^ Substitution - Nonsense	p.Q1338; C->T*º Substitution - Nonsense	p.Q1338; C->T*º Substitution - Nonsense

* Cancer Cell Line Encyclopedia (CCLE), https://www.broadinstitute.org/ccle/home

º Catalog of Somatic Mutations in Cancer (COSMIC), http://cancer.sanger.ac.uk/cosmicous

^Heterozygous mutation

	SW837 (CCL235)	SW 480 (CCL228)	SW620 (CCL 227)
c-Myc mutation	V921I	Asp258Ser	Asp258Ser

We amplified genomic c-Myc DNA from cell lines using specific primers for each exon. PCR was performed in a 20 mL final volume reaction containing: 1 µL of DNA (100 µM), 2 µL of Buffer, 12.2 µL of water, 2 µL of Magnesium, 0.4 µL of dNTPs (10 mM each), 1 µL of each primer, and 0.2 µL of Taq polymerase under the following cycling conditions: 40 cycles of 94ºC for 30 s, 55ºC for 30 s and 72ºC for 30 s. PCR products were then analyzed by automatic sequencing (3700 Abi Prism).