Plasma miR-200b in ovarian carcinoma patients: distinct pattern of pre/post-treatment variation compared to CA-125 and potential for prediction of progression-free survival

Supplementary Material

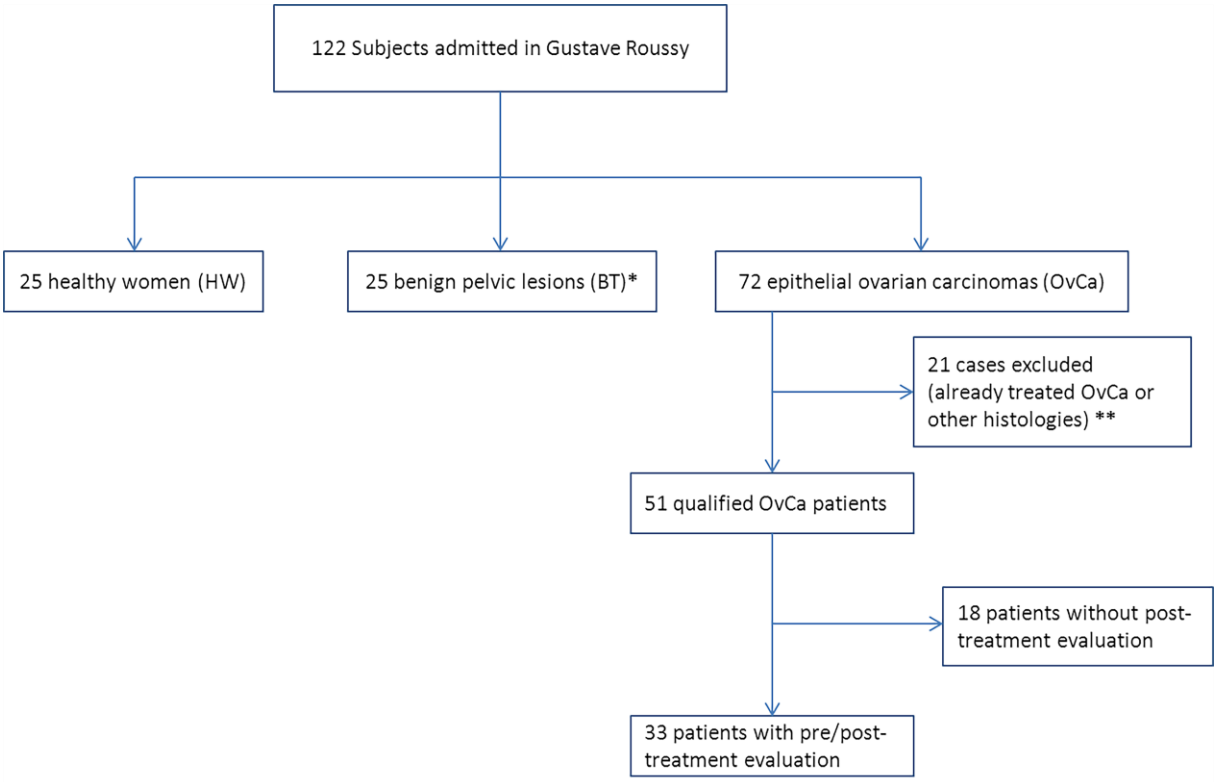

Figure 1: Flow-chart of the subjects included in the study.

*Most cases of BT were ovarian cystadenofibromas, leiomyomas, teratomas, fibrothecomas or various cysts which, after microscopical analysis of the biopsies, were shown to be non-malignant.

**Sixteen cases had received a treatment prior to the first coelioscopy or suffered a cancer within the two last years before the detection of the OvCa. Two patients were excluded, as the tumor was classified as borderline upon histopathological examination. Finally, 3 cases of ovarian tumors were proven to be the result of breast or colon cancer metastases.

Table 1: Candidate miRNAs selected for our experimental procedure.

microRNA	Material	Type of aberration	Reference
miR-21	Plasma, Serum	Increased	[1, 2]
miR-34a	Serum	Increased	[1, 3]
miR-200b	Serum	Increased	[3, 4]
miR-205	Plasma	Increased	[3, 5]

Introduction to Figure 2 - Selection of endogenous reference

Quantitative assessment of microRNAs by real-time PCR requires endogenous references to avoid biases due to RNA extraction and reverse transcription efficiency. Therefore, during preliminary studies, we investigated the potential of 3 miRNAs – hsa-miR-132-3p, hsa-miR-23a-3p and hsa-miR-191-5p – already used for normalization of plasma microRNAs in published studies [1, 6]. Our main selection criteria were their detection at a substantial and consistent level among our 3 groups of plasma samples (HW, BT, OvCa). The consistency of their concentration was evaluated by comparison of the median Ct value using the Kruskal-Wallis test. MiR-132 was rapidly eliminated because of being poorly detected in the majority of the control as well as OvCa subjects (data not shown). MiR-23a was readily detected in most plasma samples but was in fact dependent on the disease state (p-value = 0.036). In contrast, the average level of miR-191 was consistent in plasma samples from all 3 categories of donors (median values 31.91, 32.54 and 32.46 for HW, BT and OvCa respectively, p-value = 0.13). Therefore, miR-191 was chosen for data normalization (see Figure 2 below).

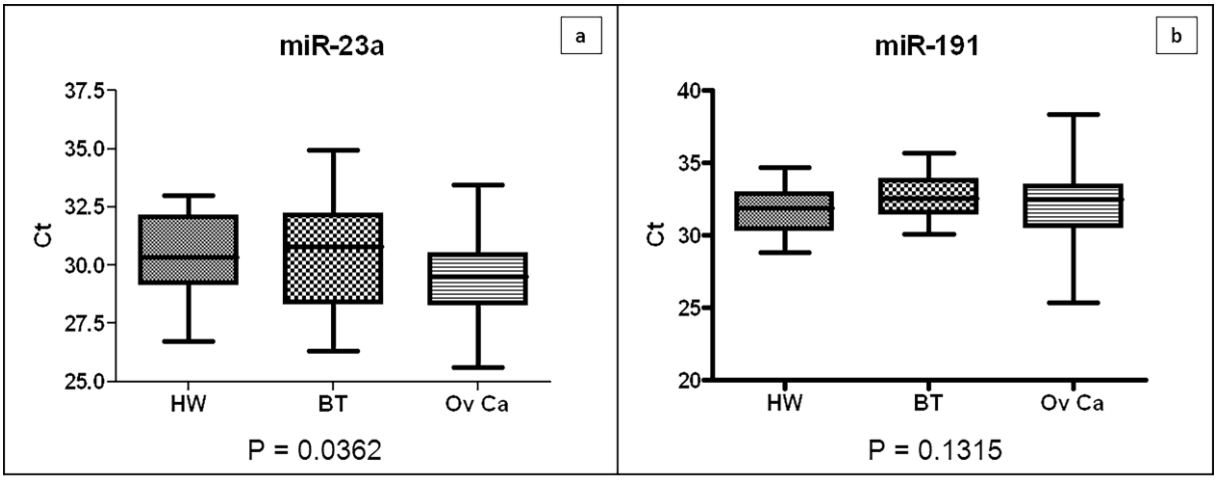

Figure 2: Comparison of miR-23a (a) and miR-191 (b) Ct values in the series of plasma samples from healthy women (HW), women with benign tumors or lesions (BT) and ovarian cancers (OvCa) (N_{HW}=25, N_{BT}=25, N_{OvCa}=51). Ct (cycle threshold) reflects the concentration of targeted miRNAs in each plasma sample (high Ct - low abundance). For miR-23a, the median values for HW, BT and OvCa were significantly different with 30.35, 30.80 and 29.49 respectively. For miR-191, they were not significantly different with 31.91, 32.54 and 32.46 respectively. The reported p-values are corrected for multiple testing. The horizontal line in the box interior represents the median.

Table 2: Real-time PCR cycle conditions

Process step	**Settings**
Polymerase Activation/Denaturation	95°C, 10 minutes
Amplification	40 cycles at 95°C for 10 seconds and 60°C for 1 minute, with a ramp-rate of 1.6°C/s
Melting Curve	Included

Table 3: Full name, accession number and Exiqon primers' reference number of each of the studied microRNAs.

microRNA	miRBase Accession Number	Exiqon Product Number
hsa-miR-21-5p	MIMAT0000076	204230
hsa-miR-23a-3p	MIMAT0000078	204772
hsa-miR-34a-5p	MIMAT0000255	204486
hsa-miR-132-3p	MIMAT0000426	204129
hsa-miR-191-5p	MIMAT0000440	204306
hsa-miR-200-3p	MIMAT0000318	206071
hsa-miR-205-5p	MIMAT0000266	204487

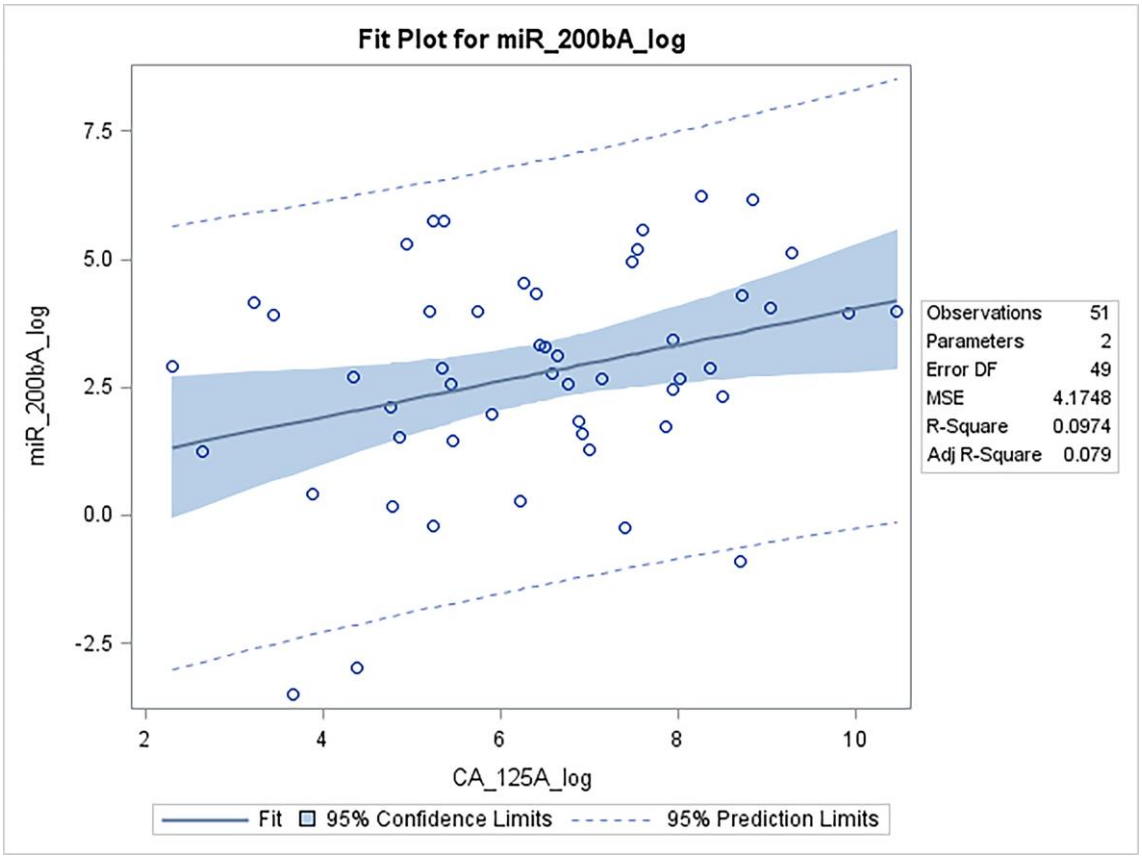

Figure 3: Plot of miR-200b versus CA-125 after log transformation: $R^2 = 0.0974$ (*N*=51).

Table 4: Correlation between CA-125, miR-200b measured prior to treatment and age, FIGO stage in ovarian cancer patients (*N*=51). Reported *p*-values (Kruskal-Wallis test) are not corrected for multiple testing.

			CA-125 Median [Q1; Q3]	miR-200b Median [Q1; Q3]
Age (years)	<65	(*N*=29)	600.0 [190.0; 1652.0]	17.7 [10.0; 53.8]
	≥65	(*N*=22)	1038.0 [119.0; 3058.0]	16.0 [1.5; 73.6]
			p-value= 0.6345	*p*-value= 0.4936
Stage FIGO	I, II	(*N*=6)	130.0 [49.0; 212.0]	48.5 [1.5; 202.7]
	III, IV	(*N*=45)	865.0 [211.0; 2858.0]	17.5 [6.3; 53.8]
			p-value= 0.0119	*p*-value=0.9301

Table 5: Individual fold changes in the concentrations of CA-125 and miR-200b in the samples collected before and after the primary treatment of OvCa patients (*N*=33).

Patient	CA-125 (U/ml)		miR-200b ($2^{-\Delta\Delta Ct}$)	
	Concentration	Fold change	Concentration	Fold change
Exo 12*	10→34	x 3.4	18.4→53.1	x 2.9
Exo 15	3058→20	1/153	14.5→6.7	1/2.2
Exo 18	90→5	1/18	13.1→97.8	x 7.5
Exo 25	2600→9.2	1/283	5.6→0.01	1/560
Exo 35	313→6	1/52	53.1→58.2	x 1.1
Exo 43	2819→41	1/69	30.3→250.0	x 8.3
Exo 45	212→11	1/19	308.0→54.6	1/6
Exo 49	211→7	1/30	17.5→24.9	x 1.4
Exo 50	190→16	1/12	308.5→420.3	x 1.4
Exo 51	184→18	1/10	53.8→3.0	1/18
Exo 54	235→8	1/29	4.3→19.5	x 4.5
Exo 58	600→30	1/20	74.6→0.04	1/1865
Exo 59	10816→26	1/416	165.7→32.4	1/5
Exo 64	2009→20	1/101	263.5→199.6	1/1.3
Exo 69	20392→57	1/358	52.4→239.6	x 4.6
Exo 72	1900→7	1/271	177.9→20.6	1/9
Exo 74	31,4→6.7	1/5	50.0→7.3	1/7
Exo 75	25→4	1/6	64.0→14.6	1/4.4
Exo 79	2858→213	1/13	11.8→311.8	x 26.4
Exo 85	8455→1600	1/5	58.2→136.8	x 2.4
Exo 103	1264→10	1/126	14.4→22.0	x 1.5
Exo 121	507→7.4	1/69	1.32→1.32	x 1
Exo 122	4269→40	1/107	17.65→1.31	x 13.5
Exo 129	3861→6	1/644	504.4→166.0	1/3.0
Exo 130	625→3.2	1/195	27.7→104.9	x 3.8
Exo 133	521.5→26	1/20	93.4→67.7	1/1.4
Exo 140	81→8	1/10	0.1→0.3	x 3
Exo 141	6900→41.6	1/166	480.0→480.6	x 1
Exo 145	39→12	1/3	0.03→13.2	x 440
Exo 153	130→31.6	1/4	4.6→48.2	x 10.5
Exo 158	49→14	1/4	1.52→0.51	1/3.0
Exo 169	141→8.2	1/17	202.7→4.4	1/46
Exo 178	1015→110.3	1/9	4,89→2.06	1/2.4

*****The patients are named according to their order of induction in the project.

References

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2. Resnick KE, Alder H, Hagan JP et al. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009; 112: 55-59.
3. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008; 110: 13-21.
4. Kan CW, Hahn MA, Gard GB et al. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. BMC Cancer 2012; 12: 627.
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6. Shapira I, Oswald M, Lovecchio J et al. Circulating biomarkers for detection of ovarian cancer and predicting cancer outcomes. Br J Cancer 2014; 110: 976-983.