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Serum Levels of MicroRNA miR-371a-3p: A Sensitive and Specific New Biomarker for Germ Cell Tumours

Klaus-Peter Dieckmann[a,†,*], *Arlo Radtke*[b,†], *Meike Spiekermann*[b,†], *Thomas Balks*[a], *Cord Matthies*[c], *Pascal Becker*[c], *Christian Ruf*[c], *Christoph Oing*[d], *Karin Oechsle*[d], *Carsten Bokemeyer*[d], *Johannes Hammel*[e], *Sebastian Melchior*[e], *Werner Wosniok*[f], *Gazanfer Belge*[b]

[a] *Department of Urology, Albertinen Krankenhaus, Hamburg, Germany;* [b] *Centre for Human Genetics, University of Bremen, Bremen, Germany;* [c] *Department of Urology, Bundeswehr Krankenhaus, Hamburg, Germany;* [d] *Department of Medical Oncology, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany;* [e] *Department of Urology, Zentralklinikum Bremen, Bremen, Germany;* [f] *Institute of Statistics, University of Bremen, Bremen, Germany*

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Abstract

Background: Clinical management of germ cell tumours (GCTs) relies on monitoring of serum tumour markers. However, the markers α-fetoprotein (AFP), the β-subunit of human chorionic gonadotropin (bHCG), and lactate dehydrogenase (LDH) are expressed in <60% of GCT cases.
Objective: To test the utility of the microRNAs (miRNAs) miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p as sensitive and specific GCT serum biomarkers.
Design, setting, and participants: Serum levels of miRNAs were measured in 166 consecutive patients with GCT before and after treatment and in 106 male controls. In the first 50 consecutive patients, all four miRNAs were measured. In the main study, only the most sensitive miRNA was further analysed.
Outcome measurements and statistical analysis: The specificity and sensitivity of the four miRNAs were studied using receiver operating characteristic curves. miRNA sensitivities were compared to those of classical markers. Statistical cross-comparisons of miRNA levels for GCT subgroups and controls were performed at various time points during treatment.
Results and limitations: Overall, miR-371a-3p performed best, with 88.7% sensitivity (95% confidence interval [CI] 82.5–93.3%) and 93.4% specificity (95% CI 86.9–97.3%) and an area under the curve of 0.94, outperforming AFP, bHCG, and LDH (combined sensitivity 50%). According to Kernel density estimation, the sensitivity and specificity were 86.3% and 92.5%, respectively. miR-371a-3p levels dropped to normal after completion of treatment. The miRNA levels correlated with treatment failure and relapse. Teratoma did not express miR-371a-3p.
Conclusions: The miRNA miR-371a-3p is a specific and sensitive novel serum GCT biomarker that accurately correlates with disease activity. Validation of this test in a large-scale prospective study is needed.
Patient summary: miR-371a-3p is a novel serum marker for germ cell tumours that is expressed by 88.7% of patients and thus is far more sensitive and specific than classical serum markers. It correlates with tumour burden and treatment results. Validation in a large patient cohort is needed.

† These authors contributed equally to this work.
* Corresponding author. Department of Urology, Albertinen Krankenhaus, Suentelstrasse 11a, D-22457 Hamburg, Germany. Tel. +40 55 882253; Fax: +40 55 882381.
E-mail address: dieckmannkp@t-online.de (K.-P. Dieckmann).

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1. Introduction

Monitoring of the serum biomarkers α-fetoprotein (AFP), the β-subunit of human chorionic gonadotropin (bHCG), and lactate dehydrogenase (LDH) is a cornerstone of clinical management of testicular germ cell tumours (GCTs) [1]. However, these markers are expressed in <60% of cases, so novel sensitive markers are needed [2]. Although many substances have been suggested as biomarkers for GCT, none have qualified for clinical use [3].

In 2011, microRNAs (miRNAs) of the clusters miR-371-3 and miR-302/367 were suggested as new serum biomarkers [4]. The miRNA molecules represent a particular class of small RNAs consisting of approximately 20 base pairs [5]. After release from the cell, these molecules remain stable in extracellular fluids [6] and can be measured by quantitative polymerase chain reaction (qPCR).

The miRNAs of the miR-371-3 and miR-302/367 clusters were originally detected in GCT tissue [7–10] and four independent pilot studies confirmed elevated serum levels [11–15]. Furthermore, circulating miRNAs of the two clusters are clearly specific for GCT because it was demonstrated that they are absent in other malignancies [16], and much higher levels of these miRNAs were found in testicular vein blood than in the peripheral circulation [17]. The goal of the present study was to further evaluate the usefulness of miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p as serum biomarkers of GCT in an unselected large patient sample. To determine whether the four miRNAs would be equally appropriate as serum biomarkers, all were tested in a preliminary study consisting of 50 GCT patients. The miRNA with the highest discriminatory power was then further evaluated in a cohort of 166 patients. We explored the utility of that miRNA as a serum biomarker by comparing its sensitivity to that of classical markers and by monitoring the response of miRNA levels to treatment.

2. Patients and methods

2.1. Patients

From June 2011 to September 2015, a total of 166 patients with GCT and 12 patients with Leydig cell tumour (LCT) who were aged 18–60 yr were prospectively enrolled from four institutions (Albertinen-Krankenhaus Hamburg, Bundeswehrkrankenhaus Hamburg, Universitätsklinikum Hamburg-Eppendorf, Klinikum Bremen-Mitte). Sixty-four participants were briefly mentioned previously in relation to a specific analysis of miRNA levels in testicular vein blood (Table 1) [12,17].

As controls, 106 male participants from the same age group were recruited (12 healthy men and 94 patients with benign scrotal conditions such as hydrocele, spermatocele, epididymitis, and varicocele) (Table 1). The first consecutive 50 GCT patients and 20 controls were participants in a preliminary study that was conducted separately. In the main study, serum samples before orchiectomy were available for 150 of the 166 GCT patients. To monitor changes in miRNA levels secondary to chemotherapy, serum samples were repeatedly collected (once per cycle) from 18 patients with clinical stage 2 (CS2) disease, nine patients with CS3 disease, and ten patients experiencing relapse. Serum aliquots were frozen and stored at −80 °C before further processing (Supplementary methods). All patients gave informed consent. Ethics approval was given by Ärztekammer Bremen (reference 301, 2011). Further clinical details are shown in Supplementary Tables 1–7.

2.2. Laboratory methods

For RNA isolation, an miRNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract total RNA from 200 μl of serum. Reverse transcription (RT) was performed using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). The RT product was preamplified, and levels of miR-371a-3p (assay 002124), miR-372-3p (assay 000560), miR-373-3p (assay 000561), and miR-367-3p (assay 000555) were measured by qPCR using a TaqMan miRNA assay (Applied Biosystems). Cycle threshold (C_t) values were normalised to miR-93-5p (assay 000432) as an internal control, and the relative quantity (RQ) was calculated using the $2^{-\Delta\Delta Ct}$ method [18]. Details of the measurement methods are described in the Supplementary methods [19].

The classical serum tumour markers AFP, bHCG, and LDH were measured according to laboratory guidelines [20]. Preoperative values were available for 139 patients.

Table 1 – Clinical data for patients with germ cell tumours, patients with Leydig cell tumours, and control subjects for the preliminary and main studies

Group	Preliminary study			Main study		
	n	Age (yr)	Tumour diameter (mm)	*n*	Age (yr)	Tumour diameter (mm)
Total GCT patients	50	37.0 (28.0–46.0)	27.0 (15.3–40.0)	166	38.5 (30.3–46.0)	29.0 (18.0–45.0)
CS1, total	40	39.0 (32.5–46.0)	25.5 (15.0–40.0)	107	40.0 (32.0–46.5)	25.0 (15.0–38.0)
CS1, seminoma	24	44.5 (38.8–48.3)	26.5 (15.0–40.0)	78 [a]	43.0 (35.0–47.8)	25.0 (15.0–38.0)
CS1, nonseminoma	16	31.0 (23.0–35.3)	25.5 (17.5–35.8)	29	30.0 (23.0–35.0)	26.5 (19.5–39.3)
CS2, total	6	27.0 (26.3–28.5)	19.5 (16.0–26.0)	38	39.5 (31.0–47.0)	33.5 (20.0–61.3)
CS2, seminoma	–	–	–	17	36.0 (31.0–46.0)	40.0 (24.0–65.8)
CS2, nonseminoma	6	27.0 (26.3–28.5)	19.5 (16.0–26.0)	21	41.0 (31.0–48.0)	30.0 (17.3–52.5)
CS3, total	4	38.5 (28.8–46.5)	52.5 (41.3–61.3)	11	36.0 (25.0–44.5)	60.0 (45.0–78.0)
CS3, seminoma	1	48.0	–	1	48.0	–
CS3, nonseminoma	3	31.0 (26.5–38.5)	60.0 (52.5–62.5)	10	33.5 (25.0–41.5)	62.5 (52.5–82.3)
Patients with relapse	–	–	–	10 [a]	29.0 (26.8–39.3)	–
Leydig cell tumours	–	–	–	12	46.0 (33.3–50.3)	–
Control subjects	20	36.0 (28.3–48.3)	–	106	38.0 (26.0–48.0)	–

GCT = germ cell tumour; CS = clinical stage. Data are presented as median (interquartile range).
[a] One patient is included in the CS1 seminoma group and in the group of relapsing patients because of relapse 2 yr after carboplatin therapy.

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