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Brief Correspondence

Integrated Expression of Circulating miR375 and miR371 to Identify Teratoma and Active Germ Cell Malignancy Components in Malignant Germ Cell Tumors

Lucia Nappi[a,b], *Marisa Thi*[b], *Nabil Adra*[c], *Robert J. Hamilton*[d], *Ricardo Leao*[e], *Jean-Michel Lavoie*[f], *Maryam Soleimani*[a], *Bernhard J. Eigl*[a], *Kim Chi*[a], *Martin Gleave*[b], *Alan So*[b], *Peter C. Black*[b], *Robert Bell*[b], *Siamak Daneshmand*[g], *Clint Cary*[c], *Timothy Masterson*[c], *Lawrence Einhorn*[c], *Craig Nichols*[h,i], *Christian Kollmannsberger*[a,*]

[a] *Department of Medicine, Medical Oncology Division, BC Cancer, Vancouver Centre, University of British Columbia, Vancouver, BC, Canada;* [b] *Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada;* [c] *Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, USA;* [d] *Department of Surgery (Urology), Princess Margaret Cancer Centre, Toronto, ON, Canada;* [e] *Hospital CUF Coimbra, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;* [f] *Department of Medical Oncology, British Columbia Cancer, Surrey Centre, Surrey, BC, Canada;* [g] *Department of Urology, University of South California, Los Angeles, CA, USA;* [h] *Testicular Cancer Commons, Beaverton, OR, USA;* [i] *SWOG Group Chairs Office, Portland, OR, USA*

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Abstract

Active germ cell malignancies express high levels of specific circulating micro-RNAs (miRNAs), including miR-371a-3p (miR371), which is undetectable in teratoma. Teratoma markers are urgently needed for theselection of patients and treatments because of the risk of malignant transformation and growing teratoma syndrome. To assess the accuracy of plasma miR375 alone or in combination with miR371 in detecting teratoma, 100 germ cell tumor patients, divided into two cohorts, were enrolled in a prospective multi-institutional study. In the discovery cohort, patients with pure teratoma and with no/low risk of harboring teratoma were compared; the validation cohort included patients with confirmed teratoma, active germ cell malignancy, or complete response after chemotherapy. The area under the receiver operating characteristic curve values for miR375, miR371, and miR371-miR375 were, respectively, 0.93 (95% confidence interval [CI]: 0.87–0.99), 0.59 (95% CI: 0.44–0.73), and 0.95 (95% CI: 0.90–0.99) in the discovery cohort and 0.55 (95% CI: 0.36–0.74), 0.74 (95% CI: 0.58–0.91), and 0.77 (95% CI: 0.62–0.93) in the validation cohort. Our study demonstrated that the plasma miR371-miR375 integrated evaluation is highly accurate to detect teratoma.

Patient summary: The evaluation of two micro-RNAs (miR375-miR371) in the blood of patients with germ cell tumors is promising to predict teratoma. This test could be particularly relevant to the identification of teratoma in patients with postchemotherapy residual disease.

* Corresponding author. Division of Medical Oncology, British Columbia Cancer, Vancouver Cancer Centre, University of British Columbia, 600 West 10th Avenue, Vancouver BC, V5Z 4E6 Canada. Tel. +1 604 877 6000; Fax: +1 604 708 2144. (C. Kollmannsberger).
E-mail address: ckollmannsberger@bccancer.bc.ca (C. Kollmannsberger).

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Micro-RNAs (miRNAs) are small noncoding RNAs involved in cell proliferation and survival [1]. Both seminoma and nonseminoma testicular germ cell tumors (GCTs) express high levels of circulating specific miRNAs, including miR-371a-3p (miR371) [2–5]. Large prospective trials evaluating the clinical utility of miR371 are underway [6]. However, miR371 is not expressed by teratoma. Surgical resection is the only curative treatment for teratoma and is recommended in nonseminoma patients presenting postchemotherapy residual disease (PCRD) >1 cm. This aggressive approach is justified by the risk of mixed active germ cell malignancies (aGCMs) and/or malignant transformation and growing teratoma syndrome, settings inadequately controlled by chemotherapy and radiation therapy. Hence, in nonseminoma patients with PCRD, predictive biomarkers to discriminate between teratoma, aGCM, and no tumor would be informative to select patients for surgery or observation [7]. As reported recently, tissue miR375 is highly expressed in teratoma and yolk sac tumor, but not in seminoma or embryonal carcinoma tissue [8–10]. However, the data regarding circulating miR375 expression by specific histology in GCT patients are limited.

The aim of the current study was to define the operating characteristics of miR375 alone or integrated with miR371 as predictive biomarkers of teratoma and aGCM.

GCT patients enrolled in the British Columbia GU Biobank were selected for the discovery cohort if they had (1) pathologically confirmed teratoma (PCT) prior to surgery, (2) clinical stage I (CSI) on surveillance (after orchiectomy; no evidence of tumor relapse), and (3) metastatic seminoma prior to chemotherapy.

In the validation cohort, metastatic nonseminoma patients from Indiana University and Princess Margaret Cancer Centre were analyzed: all were postchemotherapy patients, and had residual masses of >1 cm or radiologic complete response (CR).

The primary endpoint was to define the area under the receiver operating characteristic curve (AUC) in detecting teratoma for miR375 or miR375-miR371. Secondary endpoints were sensitivity, negative predictive value (NPV), specificity, and positive predictive value (PPV).

Blood samples were collected in cell-free DNA BCT tubes (Streck, La Vista, NE, USA); RNA was extracted from 200 μl of plasma using the miRNeasy serum/plasma kit (Qiagen, Hilden, Germany) and analyzed for miR375 and miR371 expression as reported previously [2]. The miR375 expression was correlated with the teratoma burden (Supplementary material). The Kruskal-Wallis test with Dunn' correction for multiple comparison test were used to compare the differences among the patients groups. The correlation between teratoma burden and miR375 expression was assessed by the Spearman correlation test. Logistic regression was used to calculate the AUC of miR371, miR375, or both. All tests were two sided. Significance was assumed at $p < 0.05$. Calibration plots were created for the discovery and validation sets.

Overall, 100 patients were analyzed, of whom 41 had PCT (Table 1). In the discovery cohort, 62 patients were enrolled: 20 with PCT, 27 with CSI GCT (median follow-up: 27 mo; range: 21–127), and 15 with metastatic seminoma (Supplementary Tables 1 and 2). The validation cohort included 38 postchemotherapy patients: 21 with PCT, 6 with aGCM,

Table 1 – Clinical characteristics of the teratoma patients.

Discovery cohort						Validation cohort					
Patient ID	Age (yr)	Initial histology	Teratoma diameter (mm)	Initial stage	Initial IGCCCG classification	Patient ID	Age (yr)	Initial histology	Initial stage	Initial IGCCCG classification	Teratoma diameter (mm)
				IIC	Good						
1	49	YST + EC	32	IIB	Good	21	28	SEM + EC + YST	IIB	Good	71
2	29	EC	30	IIIC	Poor	22	38	EC + YST + teratoma	IIIB	Intermediate	68
3	20	Teratoma + YST	28	IIB	Good	23	18	EC + CHORIO + YST + teratoma	IIB	Good	125
4[a]	34	EC + YST + teratoma	95	IIB	Good	24	23	EC + CHORIO + YST	IIC	Good	185
5	24	EC + SEM	5	IIIA	Good	25	32	EC + YST + teratoma	IIIA	Good	100
6	31	EC + YST + teratoma	32	IIIB	Intermediate	26	21	EC	IIA	Good	28
7	24	YST	5.5	IIIC	Poor	27	21	EC + teratoma + CHORIO + YST	IIA	Good	45
8	26	YST + teratoma + SEM	40	IIIB	Intermediate	28	16	YST + teratoma + CHORIO	IIC	Good	19
9	35	SEM + EC + teratoma	52	IIA	Good	29	27	Teratoma + EC + YST	IIB	Good	45
10	18	EC + YST	25	IIC	Good	30	23	EC + YST + teratoma	IIB	Good	18
11	48	YST + teratoma	32	IIIA	Good	31	31	EC + teratoma + YST	IIC	Intermediate	115
12	31	EC + YST + teratoma	32	IIA	Good	32	29	EC	IS	Good	40
13	19	EC + YST	25	IS	Good	33	23	EC + teratoma	IIIC	Poor	110
14	18	EC + YST	15	IIB	Good	34	28	Burned out	IIIB	Poor	60
15	30	EC + teratoma	80	IIA	Good	35	22	Teratoma + EC	IIB	Good	30
16	19	YST + teratoma + EC	25	IIIA	Intermediate	36	43	EC + teratoma + SEM + YST	IIB	Good	35
17	32	EC + YST + teratoma	1	IIA	Good	37	32	SEM + EC + teratoma + YST	IIA	Good	12
18	44	EC + YST + teratoma	25	IIIB	Poor	38	37	EC	IIB	Good	35
19	27	SEM + teratoma	40	IIB	Intermediate	39	23	EC + teratoma + YST	IIB	Intermediate	65
20	39	EC	14			40	39	Fibrosis	IIIB	Poor	17
						41	26	EC	IIB	Good	30

CHORIO = choriocarcinoma; EC = embryonal carcinoma; IGCCCG = International Germ Cell Cancer Collaborative Group; SEM = seminoma; YST = yolk sac tumor.
[a] Patients with extensive retroperitoneal and kidney involvement from teratoma.

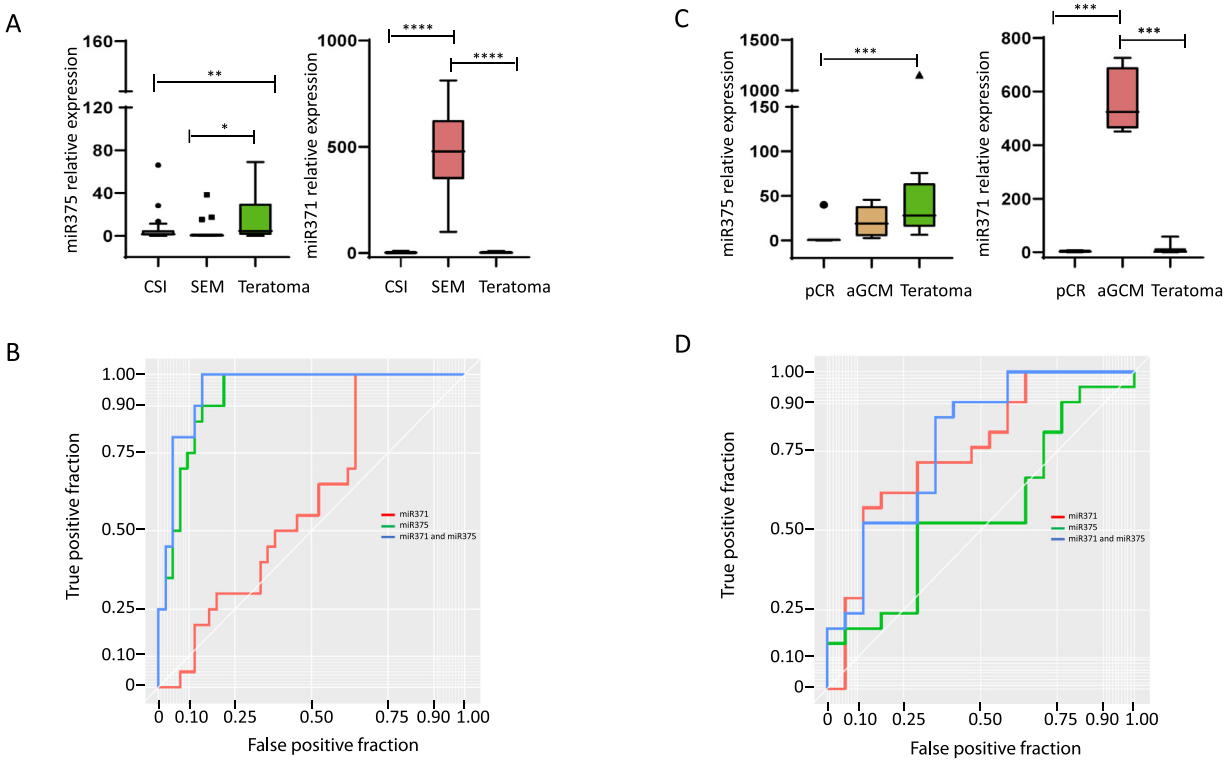

Fig. 1 – Operation characteristics of miR371 and miR375. Expression of miR375 and miR371 in patients enrolled in the (A) discovery and (C) validation cohorts. AUC of the ROC curves for miR375 (green), miR371 (red), and miR375 + mR371 (blue) in the (B) discovery and (D) validation cohort patients. Kruskal-Wallis test with Dunn's multiple comparison correction was used.
aGCM = active germ cell malignancy; AUC = area under the curve; CSI = clinical stage I; pCR = pathological complete response; ROC = receiver operating characteristic; SEM = seminoma.
*** $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.**

and 11 with CR (pathologic CR, n = 4; clinical CR, n = 7; Supplementary Fig. 1, and Supplementary Tables 3 and 4).

In the discovery cohort, plasma miR375 expression was significantly higher in patients with residual teratoma than in patients with CSI (p = 0.01) or seminoma (p = 0.04). In contrast, miR371 expression was higher in patients with seminoma and undetectable in both teratoma and CSI patients ($p < 0.0001$; Fig. 1A). Sensitivity, specificity, PPV, and NPV of miR375 for teratoma were 0.90 (95% confidence interval [CI]: 0.69–0.97), 0.81 (95% CI: 0.66–0.9), 0.69 (95% CI: 0.5–0.83), and 0.94 (95% CI: 0.81–0.98), respectively. The AUC of integrated miR371-miR375 was 0.95 (95% CI: 0.90–0.99), higher than the AUC of the individual miR375 and miR371 (0.93, 95% CI: 0.87–0.99 and 0.59, 95% CI: 0.44–0.73, respectively; Fig. 1B).

In the validation cohort, miR375 was overexpressed preoperatively in teratoma compared with patients with pathologic or clinical CR ($p < 0.001$). The expression of miR375 was higher in teratoma patients than in patients with resected aGCM; however, with the limitation of only six patients harboring aGCM, this difference was not statistically significant (p = 0.9). Conversely, preoperative miR371 was overexpressed only in patients with resected aGCM, but undetectable in patients with residual teratoma only (p = 0.0009) or with CR after chemotherapy ($p < 0.001$; Fig. 1C).

Sensitivity, specificity, PPV, and NPV of miR375 for teratoma were 0.52 (95% CI: 0.32–0.71), 0.70 (95% CI: 0.46–0.86), 0.68 (95% CI: 0.44–0.85), and 0.54 (95% CI: 0.34–0.73), respectively. The AUC of combined miR375-miR371 (0.77; 95% CI: 0.62–0.93) was higher than that of individual miR371 (0.74; 95% CI: 0.58–0.91) and miR375 (0.55; 95% CI: 0.36–0.74; Fig. 1D).

Calibration of the two sets is shown in Supplementary Figure 2. While the predicted and observed risks are close in the discovery set, the values in the validation set stray more around the diagonal line. This is likely related to the differences in clinical characteristics between the patients enrolled in the two cohorts and also to the number of individuals, which is smaller in the validation setting than in the discovery setting, leading to more noise.

The median teratoma size was 32 mm (1–185). Plasma expression of miR375 correlated significantly with residual teratoma burden (r: 0.80, 95% CI: 0.64–0.89, $p < 0.0001$; Supplementary Fig. 3).

Recent studies have confirmed the high fidelity of miR371 in identifying aGCM in GCT patients. Predictive biomarkers to identify mature teratoma would add clinical utility in common clinical scenarios [2,3]. This is particularly urgent in patients with PCRD where current clinical decisions are based on suboptimal size criteria, which expose 40–50% of patients cured with chemotherapy to

unnecessary surgery [7]. About 10% of PCRD patients harbor aGCM. Our study confirmed the high expression of miR371 in aGCM in this setting [11]. Plasma miR375 was highly expressed in patients with PCT, while miR371 was confirmed to be undetectable in teratoma-only patients. In GCTs, limited case series have described variable detectability and expression of circulating miR375 in teratoma patients [12]. The difference in teratoma expression in these studies may be explained by different patient characteristics and clinical settings, teratoma burden, different methodologies for blood collection, and miR375 analysis [9,10]. To our knowledge, our study is the largest in terms of teratoma patients enrolled (n = 41). Moreover, our results were validated in blinded, clinically and pathologically annotated specimens from independent high-volume institutions. While as a single biomarker the operating characteristics of miR375 appeared modest, simultaneous evaluation of miR371 with miR375 increased the fidelity in predicting teratoma. The presence of miR371 indicates aGCM, while the absence of miR371 and the overexpression of miR375 suggest the presence of teratoma in absence of aGCM components. The small numbers of teratoma patients, the (low) risk of occult disease in CSI patients, and the lack of available postsurgery blood samples represent the main limitations of our study.

Our study demonstrated that miR371-miR375 integrated evaluation may be clinically useful to predict GCT components, especially in patients presenting PCRD. Further investigation within larger studies for confirmation and refinement is warranted.

***Author contributions*:** Christian Kollmannsberger had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Nappi, Nichols, Kollmannsberger.
Acquisition of data: Nappi, Kollmannsberger, Black, So, Gleave, Adra, Hamilton, Leao, Cary, Lavoie, Soleimani, Eigl, Chi, Masterson, Einhorn.
Analysis and interpretation of data: Nappi, Thi, Nichols, Kollmannsberger.
Drafting of the manuscript: Nappi, Nichols, Kollmannsberger.
Critical revision of the manuscript for important intellectual content: Nappi, Nichols, Kollmannsberger, Black, So, Gleave, Adra, Hamilton, Cary, Lavoie, Soleimani, Eigl, Chi, Daneshmand, Cary, Masterson, Einhorn.
Statistical analysis: Nappi, Bell.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eururo.2020.10.024.

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