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Serum microRNA-371a-3p levels predict viable germ cell tumor in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection

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TAKE HOME MESSAGE

Circulating microRNA-371a-3p levels accurately predict viable germ cell tumor (GCT) in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection with >90% sensitivity and specificity. MiRNAs are a promising candidate biomarker to detect occult disease and change clinical practice in early-stage GCT.

Serum microRNA-371a-3p levels predict viable germ cell tumor in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection

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Serum-based biomarkers (α-fetoprotein (AFP), ß-human chorionic gonadotropin (ß-hCG), and lactate dehydrogenase (LDH) are integral in the diagnosis, management, and follow-up of patients with testicular germ cell tumors (GCTs). The clinical utility of these markers is tempered by limited sensitivity and specificity, as they are expressed in only a subset of GCTs and may be normal in the setting of early recurrence or low tumor burden (marker-negative, radiographically occult metastases). Serum microRNAs (miRNAs; miR-)—small noncoding RNAs involved with epigenetic regulation of gene expression—are emerging as candidate biomarkers for diagnosing and monitoring viable GCT, regardless of histology.[1,2] The capacity of miRNA to predict viable GCT in chemotherapy-naïve patients has not been explored. The ability to accurately personalize the decision to forego or pursue primary treatment (retroperitoneal lymph node dissection (RPLND), chemotherapy, or radiotherapy) in a cohort of patients arguably best suited for surveillance would mitigate the morbidity associated with overtreatment. Herein, we evaluate the performance characteristics of serum miRNA levels to predict viable GCT in chemotherapy-naïve patients undergoing primary RPLND.

We prospectively collected serum samples from consecutive chemotherapy-naïve patients with Type II GCT undergoing primary RPLND at our institution between 2016 and 2019 immediately prior to surgery. Preoperative cross-sectional staging imaging and conventional STMs were obtained within 10 days of surgery, and all patients were either clinical stage I or II with no radiographic evidence of distant metastases. Bilateral full-template or extended modified template (extending to the contralateral ureter above the inferior mesenteric artery) nerve-sparing RPLND was performed. RNA extraction, initial QC, and serum miRNA quantification were completed as described by Murray et al.[3] RPLND histology was classified as either benign,

viable GCT, or teratoma only. Corresponding performance characteristics (area under the curve (AUC) analysis of receiver-operator characteristic (ROC) curves, accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)) were calculated for each miRNA signature.

Our cohort consisted of 24 total patients with clinical stage I (50%) or II (50%) GCT undergoing primary RPLND. Orchiectomy histology was pure seminoma in 4 patients (16.7%), NSGCT in 18 patients (75%), and burned-out tumor in 2 patients (8.3%) with clinical stage II disease. Final RPLND histology was benign in 10 patients (41.7%), pure teratoma in 3 patients (12.5%), and viable GCT in 11 patients (45.8%). Seminoma and teratoma were identified on RPLND for patients with burned-out primary. The smallest foci of viable GCT identified were, in some cases, less than 5 mm. Four patients (16.7%) received chemotherapy post-RPLND, including one that recurred, one patient with limbic encephalitis paraneoplastic syndrome and two patients with N2 NSGCT who received adjuvant chemotherapy. Out of the prototypical 4-member putative serum miRNA panel for detection of GCT, miR-371a-3p has consistently been the most sensitive.[2,4] We also included miR-375 in our panel due to a recent report indicating high expression in teratoma tissue [5]. To determine if serum miR-371a-3p predicts the presence of viable GCT in RPLND, patients were divided into three groups based on RPLND pathology: benign, pure teratoma, or viable GCT. miR-371a-3p was the most discriminatory serum miRNA for viable GCT, exhibiting ~13,000-fold increase in expression over teratoma or benign pathology. On ROC analysis, miR-371a-3p had AUC=0.965, with sensitivity and specificity of 100% and 92%, respectively. The AUC for other serum miRNAs in predicting viable GCT were 0.874 (miR-367-3p), 0.846 (miR-372-3p), and 0.720 (miR-373-3p). Performance of miR-371a-

3p did not differ significantly from the full panel ($p > 0.05$). These serum miRNAs, including miR-375, were not predictive of pure teratoma.

In this prospective study, we found that miR-371a-3p exhibited striking performance characteristics to accurately discriminate viable GCT from benign processes or pure teratoma in patients undergoing primary RPLND with normal STMs. miR-371a-3p yielded an AUC of 0.965 and a sensitivity and specificity of 100% and 92%, respectively, at a threshold of 2-fold over normal serum. Elevations of miR-371a-3p were present in the setting of both non-teratomatous NSGCT and pure seminoma, despite normal conventional STMs. These novel results demonstrate strong performance characteristics for miR-371a-3p in the detection of occult or low-volume retroperitoneal disease. Despite being limited by small numbers and inability to sub stratify, if validated in larger cohorts, these data suggest a basis to revolutionize how patients with early stage GCT are treated. No other studies have described data regarding performance characteristics of miRNAs with pathologic confirmation of the presence or absence of small volume metastatic disease in the context of negative STMs. Two large clinical trials [AGCT1531 (NCT03067181) and SWOG-S1823] are accruing and expected to open soon, respectively, to further study the role of miRNAs in patients with early stage disease. More recently, in the largest prospective study to evaluate the performance of miR-371a-3p as a biomarker in GCT, Dieckmann et al. reported 90% sensitivity, 94% specificity, 97% PPV, 83% NPV, and an AUC of 0.966 for the primary diagnosis of malignant GCT, similar to the performance characteristics reported in our study.[2] Our study is unique in that we were able to pathologically verify the presence or absence of viable GCTs prior to clinical relapse.

Our data suggest excellent performance characteristics of miRNAs, particularly miR-371a-3p, to accurately differentiate small-volume viable GCT from benign processes or pure teratoma in patients undergoing primary RPLND, with an AUC of 0.965 and >90% sensitivity and specificity. Although none of these miRNA accurately predicted teratoma, our dataset was small and evaluation of miR-375 is still in early stages. If validated in larger cohorts, these data suggest a basis to implement precision medicine strategies in patients with early stage GCTs. Due to the early stage of this work and the technical expertise required, current clinical decision making should not rely upon miRNA assay results.

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Conflicts of interest: none

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Figure Legend

A) *Top*: Serum miR-371a-3p expression relative to normal serum. Symbols represent outliers as determined by Tukey's method. *Bottom*: ROC curve of miR-371a-3p. **B)** Summary table of results. Green box with check indicates a positive result (relative expression over threshold), blue box with dash indicates negative result. Thresholds and performance metrics are outlined in Supplementary Table 1. **C)** Graphical summary. Serum was taken immediately prior to RPLND for RNA extraction and RT-PCR. Results were compared to RPLND pathology. miR-371a-3p summarized results and performance metrics are reported below.

Illustration