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EDITORIAL

The Road Ahead for Circulating microRNAs in Diagnosis and Management of Testicular Germ Cell Tumors

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Testicular germ cell tumors (TGCTs) are the most common malignancy among young men, accounting for more average life-years lost per cancer death than any other non-pediatric malignancy [1]. These tumors can be histologically segregated into seminoma or non-seminomatous GCT (NSGCT). Serum tumor markers, including α-fetoprotein, β-human chorionic gonadotrophin, and lactate dehydrogenase, form a critical basis for diagnosis, staging, and surveillance of TGCT. Despite this, these markers have only moderate performance characteristics: combined markers are 60–85% sensitive for metastatic NSGCT and less than 50% sensitive for seminoma [2, 3]. These markers also demonstrate limited specificity, with elevation possible in the context of liver disease and hypogonadism (not uncommon during and following TGCT treatment, respectively) or other scenarios [4]. Given their utility in the diagnosis and management of TGCT, better serum markers are sorely needed.

Serum microRNAs (miRNA, miR-) have emerged as a highly sensitive and specific potential addition to the markers already used in TGCT management. Circulating miR-371a-3p, in particular, has demonstrated excellent performance in the context of testicular disease. In the largest study reported to date, serum miR-371a-3p examined immediately prior to orchiectomy yielded 90.1% sensitivity compared with less than 50% for conventional markers [5]. Similar results have been reported from other distinct research groups [6, 7]. Despite these encouraging findings, further work is necessary prior to widespread clinical use of miR-371a-3p as a serum marker of TGCT. Here, we touch on three key areas of interest to consider prior to routine clinical implementation: interlaboratory heterogeneity, the detection of minimal residual disease, and the detection of teratoma.

To address the issue of interlaboratory heterogeneity, in this issue of *Molecular Diagnosis and Therapy*, Qiangzhao et al. [8] perform a systematic review and meta-analysis of the performance of miR-371a-3p in the detection of TGCT. Six studies were identified, totalling 1835 eligible subjects, in the pre-orchiectomy setting. Heterogeneity analysis of the included reports revealed that location of the study was a significant driver of heterogeneity. This suggests that the test may perform slightly differently in various laboratory settings and cements the utility of a meta-analysis of multiple studies from different locations to assess overall assay performance. The authors report a pooled 90% sensitivity, 93% specificity, and an area under the receiver operating characteristic (ROC) curve of 0.94 for circulating miR-371a-3p. This meta-analysis indicates that the performance of serum miR-371a-3p in detecting TGCT pre-orchiectomy remains impressive, despite some interlaboratory heterogeneity.

A second area in which further work is most needed is in the context of minimal residual or occult disease. Currently,

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up to 50% of men with clinical stage I nonseminomatous disease and normal serum markers post-orchiectomy will harbor occult disease [9]. The improved sensitivity of serum miR-371a-3p could help to identify these patients, who would otherwise be overlooked. Leão et al. [6] reported that circulating miRNAs could detect the presence of retroperitoneal viable GCT elements following chemotherapy, with 100% sensitivity and 54% specificity to lesions ≥3 cm in diameter. Circulating miR-371a-3p alone performed equivalently to a panel of miR-371a-3p and three other previously identified miRNAs.

Identification of residual disease is particularly difficult in the chemotherapy-naïve setting, as mass size at computed tomography scan is unrelated to the presence of viable GCT [10]. We therefore set out to investigate whether the miRNA test was useful for the detection of retroperitoneal viable GCT prior to chemotherapy [7]. In a small cohort of patients negative for conventional markers, we found that circulating miR-371a-3p was 100% sensitive and 92% specific for any viable GCT at retroperitoneal lymph node dissection (RPLND). We were able to detect nonseminomatous lesions < 0.5 cm in diameter, supporting the highly sensitive nature of the test. Therefore, serum miR-371a-3p appears to be particularly useful for the detection of retroperitoneal disease both before and after chemotherapy.

Despite these encouraging results, both studies held a critical limitation: they examined serum miR-371a-3p immediately prior to RPLND. An essential question therefore remaining is one of timing. The half-life of serum miR-371a-3p is less than 12 h [11]. This is very favorable given the half-lives of conventional markers are much longer, such as α-fetoprotein at 5–7 days [4]. This suggests that testing for circulating miRNAs in the days following orchiectomy could permit a very early identification of microscopic (i.e., non-radiologically detectable) residual masses and patients at risk for relapse. However, a recent study by Lobo et al. [12] found no association between post-orchiectomy serum miR-371a-3p levels and risk of relapse. At relapse, serum miR-371a-3p levels were high, as reported previously. This suggests that there exists some as yet unknown optimal window of time between orchiectomy and RPLND to test for miR-371a-3p or that the assay requires further optimization regarding sensitivity in this specific setting. Future studies should seek to address both these issues.

Another area in which improvement is needed is in the detection of teratoma. The presence of post-pubertal teratoma in retroperitoneal lymph nodes requires surgical resection, as teratoma is insensitive to chemotherapy and has the potential for malignant transformation [13]. Neither conventional markers nor miR-371a-3p are sensitive for detection of the presence of teratoma. Therefore, it is challenging to differentiate between the presence of teratoma and benign processes when retroperitoneal lymph nodes are enlarged on imaging and conventional markers are all negative. Reports of high levels of miR-375 in teratoma tissue led to a hypothesis that circulating miR-375 may be a reliable teratoma marker [14]. A recent study from Nappi et al. [15] investigating the use of plasma miR-375 in combination with miR-371a-3p reported optimistic results, with an area under the ROC curve of 0.95 and 0.77 in the discovery and validation sets, respectively. However, several studies examining miR-375 alone in the context of teratoma have found no utility [7, 16, 17]. Our group confirmed these results and also found no serum miRNAs predictive for teratoma by small RNA sequencing [18]. Further work is clearly necessary to better characterize circulating markers of teratoma.

Circulating miRNAs are poised to change the way that TGCT is diagnosed, monitored, and treated. Despite their encouraging performance to date, enough questions remain to advise caution prior to routine clinical implementation. The issues of timing of the post-orchiectomy measurement to detect residual disease, as well as maximization of assay sensitivity in this setting of microscopic residual disease, have not yet been resolved, and reports on the ability of circulating miRNAs to detect teratoma are conflicting. An additional consideration is that, to date, there is no evidence that circulating miRNAs can be used to predict non-teratoma histologic subtypes. For this reason, it may be valuable to include circulating miRNA testing alongside current conventional tumor marker quantification, which can provide some insight into TGCT histology. Moreover, although miR-371a-3p clearly appears to be the 'frontrunner' from the wider panel of four miRNAs previously identified in the context of TGCTs [19], it is not yet known whether this will be the most sensitive miRNA for GCTs that arise at other anatomic sites (e.g., ovarian or intracranial). Finally, the biological underpinnings of overexpression of these miRNAs are not well understood. Ongoing research, including related clinical trials such as AGCT1531 (NCT03067181) and SWOG1823 (NCT04435756), will help to fill these gaps in knowledge and usher the use of circulating miRNA into the clinic.

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