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An update on microRNAs as potential novel therapeutic targets in testicular germ cell tumors

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SUMMARY Testicular germ cell tumors (TGCTs) are the most frequent solid malignant tumors in men 20-40 years of age and the most frequent cause of death from solid tumors in this age group. Recent studies have underscored the fact that miRNA deregulation is a feature of carcinogenesis, including TGCT development and progression. MiRNAs are a group of small noncoding RNAs that bind to the 3′-untranslated region (UTR) of the targeted mRNAs, thus causing mRNA degradation or the inhibition of its translation, regulating gene expression in a temporal and tissue-specific manner. However, few miRNAs have been found to play key roles in TGCTs; recently, other miRNAs have been identified, representing novel potential therapeutic targets.

Keywords HMGA1, microRNA, testicular germ cell tumor (TGCT), seminoma

Among solid tumors, testicular germ cell tumors (TGCTs) have the highest incidence among young men (between 20 and 34 years of age), and their incidence has increased over the past few decades. TGCTs have their origin in a blocked maturation of primordial germ cells (PGCs) (*1-3*), and more evidence has reinforced the idea that an alteration of epigenetic status is able to initiate human malignant germ cell tumors and to do so in the place of somatic mutations. This clarifies the role of both genetic susceptibility and environmental factors, known as the 'geno-environment', in TGCTs (*4-9*). About 90% of TGCTs are successfully treated with cisplatin-based chemotherapy. However, this form of therapy can lead to secondary cancers and cardiovascular disease. TGCTs are classified into two principal groups: germ cell neoplasias *in situ* (GCNIS) that are seminomas or nonseminomas (NSE), and spermatocytic tumors that are not GCNIS. NSE tumors encompass embryonal carcinoma, choriocarcinoma, yolk sac tumors (YSTs), and teratoma. TGCTs may develop from a non-invasive type of tumor called carcinoma *in situ* (CIS): microscopy reveals abnormal cells even though they are still confined inside the membrane of the seminiferous tubules (*10-14*).

MicroRNAs (miRNAs) are short non-coding RNA fragments that, by binding to the 3′UTR, are able to negatively regulate gene transcripts (*15*). Thus, recent studies have underscored the fact that miRNA deregulation is a feature of carcinogenesis, including TGCT development and progression (*16-19*). Although different miRNA signatures are associated with histological subtypes of TGCTs, various miRNAs have been found to play a key role in TGCTs. Voorhoeve *et al*. reported that two miRNAs (miR-372 and miR-373) can escape the cell cycle arrest induced by p53 (*20*). Indeed, these two miRNAs were not expressed or only slightly expressed in TGCT-derived cell lines where p53 was mutated or downregulated, indicating that miR-372 and miR-373 induce TGCT growth to elude the p53 checkpoint of the cell cycle. In this context, several data suggest that miR-372 and miR-373 may act as oncogenes in TGCTs through the inhibition of LATS2, a tumor suppressor gene (*20*). Dieckmann *et al*. confirmed that serum levels of microRNA (miR)-371a-3p (a so-called M371 test) are better than the standard markers of GCT with the same level of sensitivity and a specificity of around 90% (*21*).

Moreover, Ozata *et al*. found that PEG3 mRNA can be strongly suppressed by the action of miR-514a-3p, inducing apoptosis. In particular, levels of PEG3 expression are elevated in TGCTs where the expression of miR-514a-3p is lacking (*22*).

Recent studies have reported that the deregulation of miRNA expression in cancer cells can modify the tumor microenvironment, inducing cancer progression. However, this mechanism has yet to be elucidated in TGCTs. Recent research has found that epigenetic modifications downregulate miR-125b in

TGCT samples. Indeed, xenograft models of TGCTs indicated that miR-125b plays a key role in tumor-stroma crosstalk, underscoring its tumor suppressor role and the possibility of using miR-125b as an miRNA therapeutic (*23*).

Intriguingly, both Let-7a and miR-26a were found to be downregulated in several human cancer types, acting as tumor suppressor miRNAs (*24,25*). Moreover, these miRNAs inhibited cell proliferation and invasiveness of malignant melanoma derived-cell lines, suggesting that miR-26a and Let-7a may represent novel therapies for melanoma (*26)*. Indeed, Let-7 is able to repress several oncogenes such as MYCN, AURKB, CCNF, RRM2, MKI67, and C12orf5 in TGCTs (*27*). A previous study determined that mitotic cells (spermatogonia and primary spermatocytes) express HMGA1, while HMGA2 is highly expressed in meiotic and postmeiotic cells (secondary spermatocytes and spermatids) (*28,29*). In addition, other studies have demonstrated that the expression of HMGA1 and HMGA2 plays a key role in TGCT tumorigenesis and that the two can serve as a helpful diagnostic tool when the histological differential diagnosis is in question (*30-36*). In particular, HMGA1 protein is overexpressed in human seminomas.

Recently, De Martino and colleagues found that Let-7a and miR-26a are downregulated in human seminoma, with levels negatively correlating with *HMGA1*. In addition, they found that *HMGA1* is a target of Let-7a and miR-26a in seminomas and that Let-7a and miR-26a are able to inhibit seminoma cell growth and motility. Intriguingly, since miRNAs may act on several target transcripts that share the same microRNA responsive element (MRE) inhibitory action, a topic of great interest would be to study the transcriptomic effects of Let-7a and miR-26a overexpression in seminoma-derived cell lines using RNA-seq analysis in order to obtain a broader portrait of overall changes in levels of gene expression (*37*).

The study of the deregulated molecular pathways in TGCTs has now led to the development of successful clinical approaches. Indeed, most patients with TGCTs respond well to cisplatin-based chemotherapy. However, several patients have developed chemoresistance to first-line treatments for TGCTs. Therefore, new therapies based on novel strategies could increase the potential to treat cisplatin-resistant patients and limit adverse drug reactions. Interestingly, the ability of Let-7a and miR-26a to prevent seminoma cell growth could lead to new insights in therapeutic perspectives. In fact, modern forms of therapy may originate from the restoration of normal Let-7a and miR-26a levels in seminomas *via* the administration of synthetic miRNA oligonucleotides.

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