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Review Article

The Role of ncRNA AND miRNA in the Prognosis of Rhabdomyosarcoma

Vaia Grigoriou[1], George I. Lambrou[1,2,3,4]

[1]*Postgraduate Program "Metabolic Bones Diseases", National and Kapodistrian University of Athens, Medical School, Athens, Greece;*
[2]*Laboratory for the Research of the Musculoskeletal System "Th. Garofalidis", National and Kapodistrian University of Athens, Medical School, Athens, Greece;*
[3]*Choremeio Research Laboratory, First Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece;*
[4]*University Research Institute of Maternal and Child Health & Precision Medicine, National and Kapodistrian University of Athens, Athens, Greece*

Abstract

Rhabdomyosarcoma is an aggressive type of pediatric soft tissue cancer, characterized by the abnormal growth of skeletal muscle cells. The prognosis of patients with rhabdomyosarcoma depends on tumor subtype, tumor size and location, staging, patient age, coexisting gene mutations, and response to therapy. Despite advances in treatment modalities, the prognosis for rhabdomyosarcoma patients remains variable, and better prognostic markers are needed to guide therapeutic decisions. Non-coding RNAs and microRNAs have recently been extensively studied in cancer development and progression. The decreased expression of tumor suppressor microRNAs, such as microRNA-206, microRNA-1, microRNA-29b, microRNA-133, microRNA-28-3p, microRNA-193a, microRNA-450b-5p, microRNA-221, microRNA-324-5p, microRNA-378-3p, microRNA-26 and microRNA-7, which inhibit cancer cell multiplication, migration and invasiveness, have been associated with poor prognosis. A corresponding adverse prognosis is observed in overexpression of several oncogenic microRNAs, such as microRNA-9, microRNA-223, microRNA-486-5p and microRNA-130a/b. Overexpression of long non-coding RNA H19 in rhabdomyosarcoma, mainly embryonal, is associated with tumor progression and poor prognosis. Finally, two circular RNAs have been implicated in the pathophysiology of rhabdomyosarcoma. circ-ZNF609 regulates myoblast proliferation and has been shown to be elevated in both major subtypes of rhabdomyosarcoma. circVAMP3 has been observed to be significantly overexpressed in alveolar rhabdomyosarcoma Rh4 cells in comparison to normal myoblasts. Overexpression of both circular RNAs is associated with adverse disease progression.

Keywords: Circular RNA, Long non-coding RNA, miRNA, Non-coding RNA, Rhabdomyosarcoma

Introduction

Rhabdomyosarcoma is an infrequent but aggressive malignant tumor of the childhood and adolescence that arises from the mesenchymal cells of skeletal muscle lineage. Rhabdomyosarcoma is characterized by a high risk of relapse and metastasis, making the identification of prognostic markers to stratify patients and guide treatment decisions of vital importance[1]. Recently, non-coding RNAs have been extensively studied for their regulatory roles in cancer development and progression. Among the ncRNAs, microRNAs have been observed to regulate gene expression and evolve as potential prognostic markers in

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Corresponding author: *George I. Lambrou, First Department of Pediatrics, National and Kapodistrian University of Athens, Choremeio Research Laboratory, Thivon & Levadeias 8, 11527, Goudi, Athens, Greece*

E-mail: *glamprou@med.uoa.gr*

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rhabdomyosarcoma[2]. The purpose of the present review is to summarize the role of non-coding RNAs and microRNAs, in the prognosis of rhabdomyosarcoma.

Rhabdomyosarcoma

Although rhabdomyosarcoma accounts for less than 4% of all pediatric cancers, being the most frequent soft tissue sarcoma in children and adolescents[3]. The incidence of rhabdomyosarcoma varies by age, sex, and race/ethnicity. Incidence is highest in children between 1 and 2 years of age. The incidence is slightly higher in men than in women, higher in Caucasians than in African Americans and lower in Asians and Hispanics[4].

The pathogenesis of rhabdomyosarcoma is mostly unknown, but several genetic and environmental risk factors have been identified, that may disrupt DNA structure and increase the risk of rhabdomyosarcoma. Several genetic syndromes, characterized by mutations in tumor suppressor genes or oncogenes, along with environmental factors, such as ionizing radiation, pesticides and chemicals and previous radiotherapy, chemotherapy and bone marrow transplantation may predispose to the development of rhabdomyosarcoma[5-9].

Two major histological subtypes of rhabdomyosarcoma with distinct clinical and molecular characteristics have been identified: embryonal rhabdomyosarcoma and alveolar rhabdomyosarcoma. Embryonal rhabdomyosarcoma is the most common subtype, accounting for approximately 70% of all rhabdomyosarcoma cases, typically occuring in younger children and with a more favorable prognosis in comparison to alveolar rhabdomyosarcoma. It is rather localized, affecting the head and neck, the genitourinary system or the extremities. Histologically, embryonal rhabdomyosarcoma is characterized by spindle-shaped cells that resemble developing skeletal muscle cells and is further subdivided into three histologic subtypes: botryoid, spindle cell, and sclerosing[10-12]. In embryonal rhabdomyosarcoma, frequent mutations have been identified in genes such as PAX3 and PAX7, which encode transcription factors involved in myogenesis. On the other hand, alveolar rhabdomyosarcoma is more aggressive, with a worse prognosis, accounting for about 20-30% of rhabdomyosarcoma cases. It usually develops on the trunk or extremities and it affects older children and adolescents. Alveolar rhabdomyosarcoma is characterized by small, round, undifferentiated cells that resemble the primitive muscle cells of the alveoli[10-12]. In alveolar rhabdomyosarcoma, the hallmark genetic alteration is the presence of PAX3-FOXO1 (paired box 3-forkhead Box O1) or PAX7-FOXO1 fusion genes which promote tumorigenesis by dysregulating gene expression[13].

The main treatment methods for rhabdomyosarcoma include surgical excision, radiation therapy, and chemotherapy, often used in combination. Surgical excision aims to the complete removal of the tumor, with preservation of function and the reduction of recurrence risk[14]. Radiation therapy and chemotherapy may be applied postoperatively in order to destroy any remaining cancer cells or preoperatively to shrink the tumor and make it surgically removable[15-16]. A targeted therapy with promising results in the treatment of rhabdomyosarcoma is trabectedin, which attenuates cancer cell growth and induces apoptosis[1]. A promising immunotherapeutic approach in the management of rhabdomyosarcoma is the use of checkpoint inhibitors, such as pembrolizumab and nivolumab, which act by blocking certain proteins in rhabdomyosarcoma cells that suppress the immune system[1].

The prognosis of rhabdomyosarcoma is determined by several factors, including the location and size of the tumor, the stage of the disease, the subtype of rhabdomyosarcoma, the presence of the PAX3-FOXO1 fusion oncoprotein, response to treatment and age and the existence of comorbidities[17-19]. If the tumor has spread to nearby lymph nodes, the prognosis of rhabdomyosarcoma may be worse[20]. The prognosis is worse in older patients with comorbidities, with large tumors located in head and neck regions[18,21]. The prognosis for localized rhabdomyosarcoma is generally good, with a 5-year survival rate of approximately 70-80%[21].

Non-Coding RNAs

Non-coding RNAs constitute a diverse class of RNA molecules which are not translated into proteins but play crucial roles in regulating gene expression at the transcriptional and post-transcriptional levels. They are broadly classified into two categories based on their size: small non-coding RNAs and long non-coding RNAs. Small non-coding RNAs have typically a length of less than 200 nucleotides and include microRNAs, small interfering RNAs, and PIWI-interacting RNAs. Among these, microRNAs are the most extensively studied in the context of cancer. Some non-coding RNAs have been shown to act as molecular sponges, sequestering microRNAs and preventing them from interacting with their target messenger RNAs (mRNAs) 22.

MicroRNAs

MicroRNAs are a class of small non-coding RNAs playing a pivotal role in post-transcriptional gene regulation. They typically consist of 19-24 nucleotides and function by connecting to the 3' untranslated region (UTR) of target mRNAs, leading to mRNA degradation or translational repression. MicroRNAs are participating in diverse cellular processes, including gene expression, cell proliferation, differentiation, immune response, cell cycle regulation, DNA repair and apoptosis. Dysregulation of microRNAs has been noted in several cancer types, including rhabdomyosarcoma. In cancers, depending on their target genes, microRNAs act as oncogenes (oncomiRs) or tumor suppressors, contributing to cancer initiation, progression, and metastasis[23-25].

MicroRNAs are initially transcribed by RNA polymerase II as long primary microRNAs, which are then processed by the Drosha- DiGeorge syndrome critical region 8 (DGCR8)

complex in the nucleus to generate precursor microRNAs, whose length is about 70 nucleotides. Precursor microRNAs are subsequently transferred to the cytoplasm and further processed by the Dicer enzyme to form mature microRNAs, which are loaded onto the RNA-induced silencing complex (RISC), where they guide RISC to target mRNAs for degradation or translational repression. microRNAs act by connecting to target mRNAs through complementary base pairing, leading to mRNA degradation or translational repression, thereby regulating gene expression[26-27].

Long Non-Coding RNAs

Long non-coding RNAs are RNA molecules, with a length of more than 200 nucleotides, that do not have protein-coding potential. Through their interaction with chromatin-modifying enzymes and transcription factors, they participate in several regulatory mechanisms, such as chromatin organization, transcriptional regulation and RNA processing. They can act as scaffolds for protein complexes, decoys for miRNAs, or modulators of transcription, guiding the assembly of protein complexes at specific genomic loci[28]. Long non-coding RNAs have been shown to interact with proteins and other non-coding RNAs to form functional complexes that regulate gene expression[22]. They also have the potential to regulate the post-translational function of proteins through phosphorylations, glycosylations and acetylations, affecting growth, cell differentiation and disease pathogenesis[29]. Long non-coding RNAs have been implicated in various aspects of cancer biology, including cell proliferation and invasion and metastasis. For example, the long non-coding RNA HOTAIR (HOX antisense intergenic RNA) has been involved in the regulation of HOX genes and is associated with various types of cancer metastasis[30].

Circular RNAs

Another smaller class of non-coding RNAs are circular RNAs which are characterized by a closed-loop structure, resulting during RNA processing, along with inherent stability and abundance in cells. They act as miRNA sponges, sequestering microRNAs and blocking their interaction with target mRNAs. Circular RNAs may also interact with proteins and participate in gene regulation and signaling pathways[31-32].

MicroRNAs in Rhabdomyosarcoma

MicroRNAs play a crucial role in the regulation of myogenesis, which is the process of muscle cell formation and differentiation. Myogenesis involves the activation of muscle-specific genes, cell cycle withdrawal, and the fusion of myoblasts to form multinucleated muscle fibers. In myogenesis, microRNAs participate in various stages, influencing the expression of key genes and controlling the balance between proliferation and differentiation of myogenic precursor cells[33]. Several microRNAs are specifically expressed during myogenesis and are often referred to as "myomiRs." These include microRNA-1, microRNA-133, microRNA-206, microRNA-208, microRNA-486, and microRNA-499. Several studies have reported dysregulated myomiRs in rhabdomyosarcoma, shedding light on their potential roles in the evolution and progression of this cancer[34]. These dysregulated microRNAs may have either oncogenic or tumor-suppressive role, contributing to the heterogeneity of rhabdomyosarcoma. According to Novak et al. "since myomiRs play an important role in the differentiation of skeletal muscle tissue, their downregulation could be one of the factors responsible for the dedifferentiation of their phenotype in rhabdomyosarcoma"[35].

Oncogenic microRNAs in rhabdomyosarcoma

Some miRNAs with oncogenic properties (Onco-miRs) have been found, which are overexpressed in rhabdomyosarcoma, adversely affecting the prognosis of the disease and promoting tumorigenesis by targeting tumor suppressor genes. The **microRNA-9** family is involved in suppressing E-cadherin expression, a protein playing a vital role in rhabdomyosarcoma metastatic behavior and intercellular communication. Overexpression of microRNA-9-5p is associated with poor clinical outcome and increased likelihood of metastases. Decrease in PAX3-FOXO1 expression resulted in a reduction of microRNA-9-5p expression[36]. **MicroRNA-27a** has been found to be upregulated in the more aggressive rhabdomyosarcoma cell lines. MicroRNA-27a was shown to enhance rhabdomyosarcoma cell proliferation and tumor progression by suppressing retinoic acid α receptor (RARA) and retinoic X receptor α (RXRA)[37].

MicroRNA-183 has been found to act as an onco-miR in various types of cancer, including rhabdomyosarcoma[38]. It is significantly overexpressed in rhabdomyosarcoma. Experimental studies in microRNA-183 knockdown mice resulted in significant attenuation in cell migration via the direct stimulation of epidermal growth factor receptor 1 (*EGR1*) expression[38]. **MicroRNA-214** is known to target phosphatase and tensin homolog (PTEN), a tumor suppressor, and its upregulation may lead to increased cell survival and proliferation. High expression of microRNA-214 has been associated with poor survival in patients with rhabdomyosarcoma[39]. According to Gasparini et al. overexpression of **microRNA-223** is associated "with increased epithelial-mesenchymal transition and inflammatory signaling pathways, possibly implicating its role in aggressiveness and inflammatory pathways of rhabdomyosarcoma in the adolescents and young adults"[40]. The same study concluded that **microRNA-431** "was correlated to myogenic differentiation and muscle metabolism, yet supporting its increase in pediatric rhabdomyosarcoma"[40]. Overexpression of **microRNA-486-5p** and **microRNA-130a/b** has been associated with increased cell proliferation, invasiveness, and migration of rhabdomyosarcoma cells and a higher risk of metastasis and poorer prognosis[41-43].

Tumor-suppressive microRNAs in rhabdomyosarcoma

Conversely, certain microRNAs can have tumor suppressive actions in rhabdomyosarcoma by targeting oncogenes or genes that promote cell proliferation and metastasis.

MicroRNA-1 and **microRNA-206** are involved in promoting myogenic differentiation. They target and downregulate genes like HDAC4 (histone deacetylase 4), MET (hepatocyte growth factor receptor), CCND2 (cyclin D2), ZNF281 (zinc Finger Protein 281), SMARCD1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1) and the transcription factor PAX7, which are inhibitors of myogenic differentiation. These microRNAs are essential for muscle regeneration, by promoting the differentiation of satellite cells into myoblasts, and play a role in myoblast fusion and the repair of damaged muscle tissue by targeting the transcription factor myogenin. MicroRNA-1 and microRNA-206 have tumor suppressor properties, by promoting myogenetic differentiation, inhibiting migration and proliferation of rhabdomyosarcoma cells[44-45]. MicroRNA-1 has been studied in both types of rhabdomyosarcoma, and its downregulation has been associated with high aggressiveness and a poorer prognosis. MicroRNA-206 is frequently downregulated in rhabdomyosarcoma and has been associated with better prognosis. Its reduced expression is linked to advanced clinical stages and worse outcomes[46]. According to a study by Li et al. "overexpression of microRNA-1/206 showed a strong pro-myogenic effect in rhabdomyosarcoma cells and reduced *CCND2* transcription levels"[44]. Two experimental studies have suggested that heterotopic expression of microRNA-206 by lentiviral vectors results in cell cycle arrest and myogenic differentiation of rhabdomyosarcoma cells, suppressing cell growth in vivo, due to inhibition of oncogene expression *c-MET*[45,47].

MicroRNA-29 family members have tumor suppressor properties and have been investigated in rhabdomyosarcoma. MicroRNA-29 regulates myoblast fusion by targeting several extracellular matrix proteins, including collagens and fibrillins. Moreover, it blocks the proliferation, migration and invasion of rhabdomyosarcoma cells. These microRNAs are often downregulated in rhabdomyosarcoma due to an upregulation in the NFκB-YY1, and their decreased expression has been associated with adverse clinical outcomes. In a mouse model of rhabdomyosarcoma, microRNA-29 re-expression suppressed tumor development, suggesting a tumor-suppressor role for this microRNA[44,46].

MicroRNA-133 acts as tumor suppressive in rhabdomyosarcoma and promotes myoblast proliferation by targeting SRF (Serum Response Factor), HDAC4 and SMARCD1 and inhibiting myogenic differentiation. microRNA-133 has been noted to attenuate cell proliferation in rhabdomyosarcoma cell lines, by targeting and downregulating several genes involved in cell cycle progression. microRNA-133 inhibits the growth of rhabdomyosarcoma cells and serum levels are decreased in patients with rhabdomyosarcoma[48]. microRNA-133 play a role in myoblast fusion by targeting the transcription factor myogenin. Moreover, microRNA-133 responds to muscle stress and injury, contributing to the adaptive responses of muscle tissue. It is downregulated in rhabdomyosarcoma tissues in comparison to normal skeletal muscle tissues. Altered expression of microRNA-133 has been correlated with clinical outcomes in patients with rhabdomyosarcoma. Lower levels of microRNA-133 are often associated with more aggressive tumors and poorer prognosis. Some studies suggest that microRNA-133 may play a role in suppressing metastasis in rhabdomyosarcoma. It may do so by influencing the epithelial-mesenchymal transition, a process associated with cancer cell invasion and metastasis. The expression of microRNA-133 can be controlled by epigenetic mechanisms, including DNA methylation and histone modifications. Epigenetic silencing of microRNA-133 may contribute to its downregulation in rhabdomyosarcoma[48].

MicroRNA-378a-3p may act as a tumor suppressor in rhabdomyosarcoma and the restoration of its expression would be beneficial in patients with rhabdomyosarcoma. **microRNA-378-3p** over-expression was functionally demonstrated to suppress migration of rhabdomyosarcoma cells, induce apoptosis and cell cycle arrest through *IGF1R* (Insulin-like growth factor1 receptor) downregulation. Moreover, according to Megiorni et al. "replacement of microRNA-378a-3p induces cytoskeleton organization as well as modulation of muscle protein, such as MyoD1 (*myoblast determination protein 1*), MyoR, desmin and the myosin heavy chain"[49].

Other tumor suppressor miRNAs underexpressed in rhabdomyosarcoma are microRNA-7, microRNA-26a, microRNA-28-3p, microRNA-193a, microRNA-450b-5p, microRNA-221, microRNA-324-5p, microRNA-184, which inhibit the proliferation, migration and invasiveness of rhabdomyosarcoma cells, thus conferring an unfavorable prognosis in the progression of the disease[42,48-53]. In a study by Sun et al. "rhabdomyosarcoma growth and proliferation were significantly arrested by **microRNA-450b-5p**, strictly regulated by tumor growth factor-β1 (TGF-β1)"[52]. **MicroRNA-28-3p** is another tumor suppressive microRNA, acting on EZRIN protein and resulting in downregulating rhabdomyosarcoma cels migration and invasion, inhibition of cell adhesion to endothelial cells, attenuation of rhabdomyosarcoma proliferation, cell cycle arrest and enhancement of differentiation[51]. **MicroRNA-221** is a tumor suppressive microRNA that induces apoptosis and inhibits migration and invasion of rhabdomyosarcoma cells by targeting CCND2, CDK6 (Cell division protein kinase 6) and ERBB3 (Erb-B2 Receptor Tyrosine Kinase 3). Its downregulation suggests a worse prognosis for patients with rhabdomyosarcoma[42]. **MicroRNA-7** and **microRNA-324-5p** act on integrin α-9, resulting in inhibition of rhabdomyosarcoma cell proliferation and migration along

with decrease of tumor growth and metastasis in vivo[50]. **MicroRNA-26a** levels in the serum of pediatric patients have been suggested as a diagnostic biomarker for the prognosis of rhabdomyosarcoma[53]. **microRNA-184** has been studied as a potential prognostic marker in rhabdomyosarcoma. Its downregulation is associated with increased tumor aggressiveness and poorer outcomes.

MiRNAs in rhabdomyosarcoma subtypes

The dysregulation of specific miRNAs can also be associated with different rhabdomyosarcoma subtypes. For instance, microRNA-1 is downregulated in alveolar rhabdomyosarcoma and targets PAX7, which is involved in the fusion gene PAX7-FOXO1 characteristic of this subtype. By targeting PAX7, microRNA-1 may influence the aggressiveness of alveolar rhabdomyosarcoma. According to Rao et al. in embryonal rhabdomyosarcoma, microRNA-1 overexpression "determines the typical gene expression of muscle tissue and cell cycle arrest, while microRNA-133a decreases the expression of muscle markers"[48]. In a small study including alveolar rhabdomyosarcoma, microRNA-29 deregulation was observed, in association with an inhibition of myogenesis[46]. In another small study, microRNA-9 was noticed to be over-expressed in alveolar rhabdomyosarcoma in comparison to embryonal rhabdomyosarcoma, suggesting metastatic potentials[54].

Long non-coding RNAs in Rhabdomyosarcoma

While the role of non-coding RNAs in the prognosis of rhabdomyosarcoma is the subject of ongoing research, there is limited specific information available regarding their prognostic significance in this particular type of cancer. However, studies have highlighted the potential involvement of some ncRNAs in rhabdomyosarcoma pathogenesis and tumor behavior, which indirectly suggests their possible prognostic value. Impaired expression of non-coding RNAs may attenuate myoblast differentiation and increase proliferation of rhabdomyosarcoma cancer cells[2,55].

Long Non-coding RNAs can influence chromatin remodeling and gene expression during myogenesis. They may act as scaffolds, guides, or decoys, interacting with chromatin-modifying complexes to regulate the accessibility of muscle-specific genes[56]. Certain long non-coding RNAs can regulate the expression of myogenic transcription factors, such as MyoD and Myogenin, by modulating their chromatin accessibility or acting as transcriptional co-regulators[57]. Long Non-coding RNAs may function as ceRNAs (competitive endogenous RNAs), sequestering miRNAs and preventing them from connecting to their target mRNAs. This interaction influences the expression of target genes involved in myogenesis. Some long non-coding RNAs participate in the regulation of function, proliferation, differentiation and self-renewal of muscle satellite cells, which are crucial for muscle regeneration[58-59].

Several long non-coding RNAs have been implicated in rhabdomyosarcoma pathogenesis, and their expression has been associated with clinical outcomes. Long Non-coding RNAs play a crucial role in the differentiation of striated muscle fibers[60]. For example, the long non-coding RNA H19 has been found to be suppressed in rhabdomyosarcoma, in comparison to normal muscle tissue, and is associated with tumor aggressiveness and poor prognosis[61]. H19 may promote tumorigenesis by acting as a competing endogenous RNA that sequesters microRNA-675, thereby relieving the suppression of its target genes. Its overexpression has been associated to increased cell proliferation and invasiveness, with undesirable patho-anatomical features. Long non-coding RNA H19 promotes tumor development, invasion and metastasis through various mechanisms, including interaction with other regulatory molecules[62].

MYCNOS (MYCN opposite strand) is a long non-coding RNA that is often associated with the MYCN oncogene. MYCN plays a critical role in various cancers and MYCNOS is transcribed from the opposite strand of the MYCN gene. In rhabdomyosarcoma, MYCN overexpression contributes to cell proliferation, where its transcription is driven by PAX3-FOXO1[63-64]. According to O' Brien et al. "the long non-coding RNA *MYCNOS-01* and *MYCNOS-02* on the antisense strand of *MYCN* were found to upregulate MYCN protein levels in rhabdomyosarcoma"[63]. In alveolar rhabdomyosarcoma, which often carries the PAX3-FOXO1 fusion gene, long non-coding RNAs (lncRNAs) derived from this fusion have been implicated in tumorigenesis and prognosis.

Some long non-coding RNAs act as precursors of microRNAs involved in the process of myogenesis. The long non-coding RNA *Linc-MD1* generates microRNA-206 and microRNA-133b suggesting a role in muscle differentiation and a potential contribution in rhabdomyosarcoma differentiation[22]. Emerging evidence suggests that long non-coding RNAs may take part in regulating metastasis in rhabdomyosarcoma. For example, the lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) has been found to promote metastasis in rhabdomyosarcoma by regulating the expression of genes involved in cell migration and invasion.

Circular RNAs in Rhabdomyosarcoma

Some circularRNAs were observed to play a role in modulating myoblast differentiation. For example, Wei et al found that "circLMO7 has the functional capacity to act as an endogenous antagonist for microRNA-378a-3p, which is involved in muscle development in mammals". Authors observed that "its overexpression stimulates proliferation and suppresses differentiation of primary myoblasts in cattle"[65]. On the other hand, a study by Li et al concluded that "circFUT10 promotes myoblast differentiation but reduces their proliferation rate"[66].

Two circular RNAs have been found to participate in the pathogenesis of rhabdomyosarcoma. Circ-ZNF609 regulates myoblast proliferation and has been shown to be

elevated in both major subtypes of rhabdomyosarcoma[67]. In a recent study by Rossi et al. authors found that "circVAMP3 is significantly overexpressed in Rh4 cells of alveolar rhabdomyosarcoma compared to normal myoblasts"[68].

Challenges

While the potential of non-coding RNAs, including microRNAs and long non-codingRNAs, as prognostic markers in rhabdomyosarcoma is promising, several challenges need to be addressed for their clinical translation. One of the challenges in utilizing non-coding RNAs as prognostic markers is the lack of standardized assays for their detection and quantification. Standardized protocols and techniques are essential to ensure reproducibility and comparability of results across different studies and clinical settings. Prognostic markers, including non-coding RNAs, should be integrated with other clinical and molecular factors to create comprehensive prognostic models. Combining non-coding RNA expression profiles with known risk factors, such as tumor stage and histology, may improve the accuracy of risk stratification. Many studies investigating non-coding RNAs as prognostic markers in rhabdomyosarcoma have been conducted on relatively small patient cohorts. Larger, multi-institutional studies are needed to validate the prognostic value of specific non-coding RNAs rigorously. While associations between non-coding RNA expression and clinical outcomes are important, understanding the underlying functional mechanisms is crucial for the development of targeted therapies. Functional studies exploring how specific non-coding RNAs modulate the biology of rhabdomyosarcoma cells are necessary.

Conclusions

Rhabdomyosarcoma is a challenging pediatric cancer with variable clinical outcomes, emphasizing the need for better prognostic markers and therapeutic approaches. The prognosis of patients with rhabdomyosarcoma depends on tumor subtype, tumor size and location, staging, patient age, coexisting gene mutations, and response to therapy. Non coding RNAs, particularly microRNAs and long non-coding RNAs, have emerged as promising candidates for improving our understanding of the prognosis of rhabdomyosarcoma.

MicroRNAs, with their roles in post-transcriptional gene regulation, have been extensively studied in rhabdomyosarcoma. Dysregulated microRNAs have been associated with clinical outcomes, including survival and treatment response. Additionally, some microRNAs have shown potential as therapeutic targets, either through replacement or inhibition strategies. The decreased expression of tumor suppressor microRNAs, such as microRNA-206, microRNA-1, microRNA-29b, microRNA-133, microRNA-28-3p, microRNA-193a, microRNA-450b-5p, microRNA-221, microRNA-324-5p, microRNA-378-3p, microRNA-26 and microRNA-7, which inhibit cancer cell proliferation, migration and invasiveness, have been associated with poor prognosis. A corresponding adverse prognosis is observed in overexpression of some oncogenic microRNAs, such as microRNA-9, microRNA-223, microRNA-486-5p and microRNA-130a/b.

Long non-coding RNAs, although less explored, are gaining recognition for their roles in rhabdomyosarcoma and their prognostic value. Targeting dysregulated long non-coding RNAs, especially those involved in competing endogenous RNA networks, represents an emerging therapeutic avenue. Overexpression of long non coding RNA H19 in rhabdomyosarcoma, mainly embryonal, is associated with tumor progression and poor prognosis. Finally, the specific non-coding RNAs involved and their prognostic value may vary according to the histological subtype of rhabdomyosarcoma.

Despite the progress made, several challenges remain, including the standardization of assays, validation in larger patient cohorts, and the development of efficient delivery systems for non-coding RNA-based therapies. Furthermore, the integration of non-coding RNA profiles with other clinical and molecular factors is crucial for enhancing risk stratification in rhabdomyosarcoma.

In conclusion, the investigation of non-coding RNAs, particularly microRNAs and long non-coding RNAs, in the context of rhabdomyosarcoma prognosis is a promising area of research that holds the potential to improve outcomes for patients with rhabdomyosarcoma. Regarding the role of non-coding RNAs, current knowledge about rhabdomyosarcoma prognosis is based on a limited number of studies, and more research is needed to establish strong associations. Future studies should focus on addressing the challenges in non-coding RNA-based therapies and further unraveling the complex regulatory networks that govern rhabdomyosarcoma pathogenesis. Ultimately, a comprehensive understanding of non-coding RNAs in rhabdomyosarcoma may lead to more precise and effective treatment strategies for this rare and aggressive cancer. Further high-quality research is needed to fully understand the role of non-coding RNAs in the prognosis of rhabdomyosarcoma.

Authors' contributions

VG: Drafted the manuscript, reviewed literature. GIL: Proof-edited the manuscript, reviewed the manuscript and gave final permission for publication. All authors read and approved the manuscript.

References

1. Skapek SX, Ferrari A, Gupta AA, Lupo PJ, Butler E, Shipley J, et al. Rhabdomyosarcoma. Nat Rev Dis Primers. 2019; 5: 1.
2. Ramadan F, Saab R, Hussein N, Clézardin P, Cohen PA, Ghayad SE. Non-coding RNA in rhabdomyosarcoma progression and metastasis. Front Oncol. 2022; 12: 971174.
3. Sun X, Guo W, Shen JK, Mankin HJ, Hornicek FJ, Duan Z. Rhabdomyosarcoma: Advances in Molecular and Cellular Biology. Sarcoma. 2015; 2015: 232010.

4. Amer KM, Thomson JE, Congiusta D, Dobitsch A, Chaudhry A, Li M, et al. Epidemiology, Incidence, and Survival of Rhabdomyosarcoma Subtypes: SEER and ICES Database Analysis. J Orthop Res. 2019; 37: 2226-2230.
5. Smith AC, Squire JA, Thorner P, Zielenska M, Shuman C, Grant R, et al. Association of alveolar rhabdomyosarcoma with the Beckwith-Wiedemann syndrome. Pediatr Dev Pathol. 2001; 4: 550-558.
6. Trahair T, Andrews L, Cohn RJ. Recognition of Li Fraumeni syndrome at diagnosis of a locally advanced extremity rhabdomyosarcoma. Pediatr Blood Cancer. 2007; 48: 345-348.
7. Yang P, Grufferman S, Khoury MJ, Schwartz AG, Kowalski J, Ruymann FB, et al. Association of childhood rhabdomyosarcoma with neurofibromatosis type I and birth defects. Genet Epidemiol. 1995; 12: 467-474.
8. Isiklar I, Libshitz HI. Radiation-induced rhabdomyosarcoma. AJR Am J Roentgenol. 1996; 166: 217-218.
9. Martin-Giacalone BA, Weinstein PA, Plon SE, Lupo PJ. Pediatric Rhabdomyosarcoma: Epidemiology and Genetic Susceptibility. J Clin Med. 2021; 10.
10. Barr FG. Molecular genetics and pathogenesis of rhabdomyosarcoma. J Pediatr Hematol Oncol. 1997; 19: 483-491.
11. Gallego Melcón S, Sánchez de Toledo Codina J. Molecular biology of rhabdomyosarcoma. Clin Transl Oncol. 2007; 9: 415-419.
12. Parham DM, Barr FG. Classification of rhabdomyosarcoma and its molecular basis. Adv Anat Pathol. 2013; 20: 387-397.
13. Sorensen PH, Lynch JC, Qualman SJ, Tirabosco R, Lim JF, Maurer HM, et al. PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. J Clin Oncol. 2002; 20: 2672-2679.
14. Dombrowski ND, Wolter NE, Robson CD, Kawai K, Irace AL, Vargas SO, et al. Role of Surgery in Rhabdomyosarcoma of the Head and Neck in Children. Laryngoscope. 2021; 131: E984-E992.
15. Mandeville HC. Radiotherapy in the Management of Childhood Rhabdomyosarcoma. Clin Oncol (R Coll Radiol). 2019; 31: 462-470.
16. Zhao R, Yu X, Feng Y, Wang J, Chen Y, Mao Y, et al. The survival benefit of radiotherapy in localized primary adult rhabdomyosarcoma. Asia Pac J Clin Oncol. 2020; 16: 266-272.
17. Curry SD, Jiang ZY, Jain KS. Population-based survival of pediatric rhabdomyosarcoma of the head and neck over four decades. Int J Pediatr Otorhinolaryngol. 2021; 142: 110599.
18. Yang L, Takimoto T, Fujimoto J. Prognostic model for predicting overall survival in children and adolescents with rhabdomyosarcoma. BMC Cancer. 2014; 14: 654.
19. Kubo T, Shimose S, Fujimori J, Furuta T, Ochi M. Prognostic value of PAX3/7-FOXO1 fusion status in alveolar rhabdomyosarcoma: Systematic review and meta-analysis. Crit Rev Oncol Hematol. 2015; 96: 46-53.
20. Lobeck I, Dupree P, Karns R, Rodeberg D, von Allmen D, Dasgupta R. Quality assessment of lymph node sampling in rhabdomyosarcoma: A surveillance, epidemiology, and end results (SEER) program study. J Pediatr Surg. 2017; 52: 614-617.
21. Lee RJ, Lee KK, Lin T, Arshi A, Lee SA, Christensen RE. Rhabdomyosarcoma of the head and neck: impact of demographic and clinicopathologic factors on survival. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017; 124: 271-279.
22. Yoon JH, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol. 2014; 34: 9-14.
23. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6: 259-269.
24. Shruti K, Shrey K, Vibha R. Micro RNAs: tiny sequences with enormous potential. Biochemical and biophysical research communications. 2011; 407: 445-449.
25. Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev. 2003; 67: 657-685.
26. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev. 2016; 96: 1297-1325.
27. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009; 10: 155-159.
28. Mishra K, Kanduri C. Understanding Long Noncoding RNA and Chromatin Interactions: What We Know So Far. Noncoding RNA. 2019; 5.
29. Zhang X, Wang W, Zhu W, Dong J, Cheng Y, Yin Z, et al. Mechanisms and Functions of Long Non-Coding RNAs at Multiple Regulatory Levels. International journal of molecular sciences. 2019; 20.
30. Rajagopal T, Talluri S, Akshaya RL, Dunna NR. HOTAIR LncRNA: A novel oncogenic propellant in human cancer. Clinica chimica acta; international journal of clinical chemistry. 2020; 503: 1-18.
31. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. Nature reviews Clinical oncology. 2022; 19: 188-206.
32. Zang J, Lu D, Xu A. The interaction of circRNAs and RNA binding proteins: An important part of circRNA maintenance and function. J Neurosci Res. 2020; 98: 87-97.
33. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006; 6: 857-866.
34. Bersani F, Lingua MF, Morena D, Foglizzo V, Miretti S, Lanzetti L, et al. Deep Sequencing Reveals a Novel miR-22 Regulatory Network with Therapeutic Potential in Rhabdomyosarcoma. Cancer research. 2016; 76: 6095-6106.
35. Novák J, Vinklárek J, Bienertová-Vašků J, Slabý O. MicroRNAs involved in skeletal muscle development and their roles in rhabdomyosarcoma pathogenesis. Pediatr Blood Cancer. 2013; 60: 1739-1746.
36. Missiaglia E, Shepherd CJ, Aladowicz E, Olmos D, Selfe J, Pierron G, et al. MicroRNA and gene co-expression networks characterize biological and clinical behavior of rhabdomyosarcomas. Cancer Lett. 2017; 385: 251-260.
37. Tombolan L, Zampini M, Casara S, Boldrin E, Zin A, Bisogno G, et al. MicroRNA-27a Contributes to Rhabdomyosarcoma Cell Proliferation by Suppressing RARA and RXRA. PloS one. 2015; 10: e0125171.
38. Sarver AL, Li L, Subramanian S. MicroRNA miR-183 functions as an oncogene by targeting the transcription factor EGR1 and promoting tumor cell migration. Cancer research. 2010; 70: 9570-9580.
39. Huang HJ, Liu J, Hua H, Li SE, Zhao J, Yue S, et al. MiR-214 and N-ras regulatory loop suppresses rhabdomyosarcoma cell growth and xenograft tumorigenesis. Oncotarget. 2014; 5: 2161-2175.
40. Gasparini P, Fortunato O, De Cecco L, Casanova M, Iannó MF, Carenzo A, et al. Age-Related Alterations in Immune Contexture Are Associated with Aggressiveness in Rhabdomyosarcoma. Cancers (Basel). 2019; 11.
41. Ghamloush F, Ghayad SE, Rammal G, Fahs A, Ayoub AJ, Merabi Z, et al. The PAX3-FOXO1 oncogene alters exosome miRNA content and leads to paracrine effects mediated by exosomal miR-486. Scientific reports. 2019; 9: 14242.
42. Hanna JA, Garcia MR, Lardennois A, Leavey PJ, Maglic D, Fagnan A, et al. PAX3-FOXO1 drives miR-486-5p and represses miR-221 contributing to pathogenesis of alveolar rhabdomyosarcoma. Oncogene. 2018; 37: 1991-2007.
43. Pan Y, Li J, Lou S, Chen W, Lin Y, Shen N, et al. Down-Regulated miR-130a/b Attenuates Rhabdomyosarcoma Proliferation via PPARG.

Front Mol Biosci. 2021; 8: 766887.
44. Li L, Sarver AL, Alamgir S, Subramanian S. Downregulation of microRNAs miR-1, -206 and -29 stabilizes PAX3 and CCND2 expression in rhabdomyosarcoma. Lab Invest. 2012; 92: 571-583.
45. Yan D, Dong Xda E, Chen X, Wang L, Lu C, Wang J, et al. MicroRNA-1/206 targets c-Met and inhibits rhabdomyosarcoma development. The Journal of biological chemistry. 2009; 284: 29596-29604.
46. Wang H, Garzon R, Sun H, Ladner KJ, Singh R, Dahlman J, et al. NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. Cancer Cell. 2008; 14: 369-381.
47. Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, et al. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. J Clin Invest. 2009; 119: 2366-2378.
48. Rao PK, Missiaglia E, Shields L, Hyde G, Yuan B, Shepherd CJ, et al. Distinct roles for miR-1 and miR-133a in the proliferation and differentiation of rhabdomyosarcoma cells. FASEB J. 2010; 24: 3427-3437.
49. Megiorni F, Cialfi S, McDowell HP, Felsani A, Camero S, Guffanti A, et al. Deep Sequencing the microRNA profile in rhabdomyosarcoma reveals down-regulation of miR-378 family members. BMC Cancer. 2014; 14: 880.
50. Molist C, Navarro N, Giralt I, Zarzosa P, Gallo-Oller G, Pons G, et al. miRNA-7 and miRNA-324-5p regulate alpha9-Integrin expression and exert anti-oncogenic effects in rhabdomyosarcoma. Cancer Lett. 2020; 477: 49-59.
51. Skrzypek K, Nieszporek A, Badyra B, Lasota M, Majka M. Enhancement of myogenic differentiation and inhibition of rhabdomyosarcoma progression by miR-28-3p and miR-193a-5p regulated by SNAIL. Mol Ther Nucleic Acids. 2021; 24: 888-904.
52. Sun MM, Li JF, Guo LL, Xiao HT, Dong L, Wang F, et al. TGF-β1 suppression of microRNA-450b-5p expression: a novel mechanism for blocking myogenic differentiation of rhabdomyosarcoma. Oncogene. 2014; 33: 2075-2086.
53. Tombolan L, Millino C, Pacchioni B, Cattelan M, Zin A, Bonvini P, et al. Circulating miR-26a as Potential Prognostic Biomarkers in Pediatric Rhabdomyosarcoma. Frontiers in genetics. 2020; 11: 606274.
54. Armeanu-Ebinger S, Herrmann D, Bonin M, Leuschner I, Warmann SW, Fuchs J, et al. Differential expression of miRNAs in rhabdomyosarcoma and malignant rhabdoid tumor. Experimental cell research. 2012; 318: 2567-2577.
55. Gasparini P, Ferrari A, Casanova M, Limido F, Massimino M, Sozzi G, et al. MiRNAs as Players in Rhabdomyosarcoma Development. International journal of molecular sciences. 2019; 20.
56. Wang S, Zuo H, Jin J, Lv W, Xu Z, Fan Y, et al. Long noncoding RNA Neat1 modulates myogenesis by recruiting Ezh2. Cell Death Dis. 2019; 10: 505.
57. Zhou L, Sun K, Zhao Y, Zhang S, Wang X, Li Y, et al. Linc-YY1 promotes myogenic differentiation and muscle regeneration through an interaction with the transcription factor YY1. Nat Commun. 2015; 6: 10026.
58. Chen R, Lei S, Jiang T, Zeng J, Zhou S, She Y. Roles of lncRNAs and circRNAs in regulating skeletal muscle development. Acta Physiol (Oxf). 2020; 228: e13356.
59. Wang S, Jin J, Xu Z, Zuo B. Functions and Regulatory Mechanisms of lncRNAs in Skeletal Myogenesis, Muscle Disease and Meat Production. Cells. 2019; 8.
60. Jin JJ, Lv W, Xia P, Xu ZY, Zheng AD, Wang XJ, et al. Long noncoding RNA SYISL regulates myogenesis by interacting with polycomb repressive complex 2. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: E9802-E9811.
61. Casola S, Pedone PV, Cavazzana AO, Basso G, Luksch R, d'Amore ES, et al. Expression and parental imprinting of the H19 gene in human rhabdomyosarcoma. Oncogene. 1997; 14: 1503-1510.
62. Yoshimizu T, Miroglio A, Ripoche MA, Gabory A, Vernucci M, Riccio A, et al. The H19 locus acts in vivo as a tumor suppressor. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 12417-12422.
63. O'Brien EM, Selfe JL, Martins AS, Walters ZS, Shipley JM. The long non-coding RNA MYCNOS-01 regulates MYCN protein levels and affects growth of MYCN-amplified rhabdomyosarcoma and neuroblastoma cells. BMC Cancer. 2018; 18: 217.
64. Mercado GE, Xia SJ, Zhang C, Ahn EH, Gustafson DM, Laé M, et al. Identification of PAX3-FKHR-regulated genes differentially expressed between alveolar and embryonal rhabdomyosarcoma: focus on MYCN as a biologically relevant target. Genes Chromosomes Cancer. 2008; 47: 510-520.
65. Wei X, Li H, Yang J, Hao D, Dong D, Huang Y, et al. Circular RNA profiling reveals an abundant circLMO7 that regulates myoblasts differentiation and survival by sponging miR-378a-3p. Cell Death Dis. 2017; 8: e3153.
66. Li H, Yang J, Wei X, Song C, Dong D, Huang Y, et al. CircFUT10 reduces proliferation and facilitates differentiation of myoblasts by sponging miR-133a. Journal of cellular physiology. 2018; 233: 4643-4651.
67. Rossi F, Legnini I, Megiorni F, Colantoni A, Santini T, Morlando M, et al. Circ-ZNF609 regulates G1-S progression in rhabdomyosarcoma. Oncogene. 2019; 38: 3843-3854.
68. Rossi F, Centrón-Broco A, Dattilo D, Di Timoteo G, Guarnacci M, Colantoni A, et al. CircVAMP3: A circRNA with a Role in Alveolar Rhabdomyosarcoma Cell Cycle Progression. Genes (Basel). 2021; 12.