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Deciphering the Role of Epigenetics in Osteosarcoma Progression: Pathways to Novel Therapeutics

Md Munzur Rahman[1]*, Parisa Yazdizadeh Shotorbani[2]
[1] *Department of Orthopaedics Surgery, Rajshahi Medical College, Rajshahi*
[2] *Department of Cell & Molecular Biology University of Houston, TX 77004, USA*

ABSTRACT ***Background:*** Osteosarcoma (OS) is a highly aggressive pediatric bone cancer with poor prognosis, especially in metastatic cases. Epigenetic modifications play a pivotal role in OS progression and metastasis. ***Objective:*** This study aims to investigate the role of epigenetic changes in OS and explore their potential as therapeutic targets for novel treatments. ***Method:*** A cohort of 112 OS patients from the Department of Orthopaedic Surgery, Rajshahi Medical College Hospital, was analyzed from June 2022 to June 2024. DNA methylation, histone modification, and microRNA expression profiles were assessed using PCR, chromatin immunoprecipitation, and RNA sequencing. The methylation status of tumor suppressor genes (p16INK4a, RASSF1A) was correlated with clinical outcomes. Data were analyzed using statistical methods, including Chi-square tests, logistic regression, and survival analysis. ***Results:*** Aberrant DNA methylation was observed in 67% of cases, with a significant association between methylation of p16INK4a and poor prognosis ($p<0.01$). Histone modification analysis revealed a 55% overexpression of histone deacetylases (HDACs) in OS tissues, correlating with high tumor grade ($p<0.05$). MicroRNA profiling showed that miR-21 was overexpressed in 72% of patients, while miR-34a was significantly downregulated in 58%. Logistic regression identified DNA methylation of p16INK4a as a key risk factor for metastasis (OR=2.3, 95% CI: 1.5–3.2). Kaplan-Meier survival analysis demonstrated that patients with hypermethylated p16INK4a had a significantly lower 5-year survival rate of 35%, compared to 60% in those with normal methylation patterns ($p<0.01$). A multivariate Cox regression model revealed that the combination of DNA methylation status and HDAC overexpression was an independent prognostic factor for worse overall survival (HR=2.5, 95% CI: 1.8–3.4). ***Conclusions:*** Epigenetic alterations, including DNA methylation, histone modification, and microRNA dysregulation, are critical in osteosarcoma progression. These findings support the potential of targeting epigenetic mechanisms for novel therapeutic strategies.
Keywords: Osteosarcoma, Epigenetics, DNA Methylation, Histone Modification, MicroRNA.

∗**Corresponding author**: Md Munzur Rahman

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INTRODUCTION

Osteosarcoma (OS), a primary malignant bone tumor, predominantly affects children and adolescents, representing one of the most aggressive cancers within the pediatric oncology landscape. Despite advancements in surgical and chemotherapeutic interventions, the prognosis for OS patients remains poor, especially for those with metastatic or relapsed disease [1]. Traditional treatment regimens, including chemotherapy and limb-salvage surgery, often come with severe side effects and limited efficacy in advanced stages, underscoring the need for more targeted and effective therapeutic

strategies. In this context, epigenetic regulation has emerged as a crucial factor in the pathogenesis of osteosarcoma, offering novel insights into its progression and potential therapeutic approaches. Epigenetic modifications, which include DNA methylation, histone modification, and non-coding RNA regulation, play pivotal roles in regulating gene expression without altering the underlying genetic code. Recent research has revealed how these modifications contribute to the onset, progression, and metastasis of OS, suggesting that targeting the epigenome could hold promise for novel, more effective treatments [2]. Epigenetics refers to changes in gene activity that do not involve alterations to the DNA sequence itself but rather to modifications of the chromatin structure and its associated proteins. These modifications serve as key regulators of cellular processes such as differentiation, growth, and apoptosis, which are often dysregulated in [3]. In OS, these epigenetic alterations are thought to disrupt the normal balance between cell proliferation and death, driving uncontrolled tumor growth and metastasis [4]. For instance, hypermethylation of tumor suppressor genes such as *p16INK4a* and *RASSF1A* has been implicated in OS pathogenesis, leading to the silencing of critical pathways that prevent tumorigenesis. Furthermore, aberrant histone modifications and the overexpression of non-coding RNAs, particularly microRNAs, have been shown to influence OS progression by modulating gene expression networks involved in cell cycle regulation, apoptosis, and epithelial-mesenchymal transition (EMT).

DNA methylation, the addition of a methyl group to cytosine residues in CpG dinucleotides, is one of the most extensively studied epigenetic modifications in cancer. In osteosarcoma, alterations in DNA methylation patterns have been shown to silence key tumor suppressor genes, contributing to the initiation and progression of the disease. Several studies have identified specific genes whose methylation status correlates with poor prognosis in OS patients, such as *CDKN2A*, *TP53*, and *BRCA1*. These findings highlight the potential for DNA methylation as a biomarker for early diagnosis and prognosis in OS, as well as a target for epigenetic therapies aimed at reversing aberrant methylation patterns. The development of small molecules that can target DNA methyltransferases (DNMTs), the enzymes responsible for the addition of methyl groups, has opened up new avenues for therapeutic intervention in OS [5]. Histone modifications are another critical aspect of epigenetic regulation in OS. Histones, the proteins around which DNA is wrapped to form chromatin, undergo a wide range of post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications influence chromatin structure and accessibility, thereby regulating gene expression. In osteosarcoma, abnormal histone modification patterns have been observed, which contribute to the silencing of tumor suppressor genes and the activation of oncogenes. For example, the dysregulation of histone acetylation and methylation at the *TP53* locus has been linked to OS progression, with reduced acetylation leading to the repression of *TP53* expression, thereby promoting tumor growth [6]. Additionally, the overexpression of histone deacetylases (HDACs) in OS cells has been associated with poor patient outcomes, suggesting that HDAC inhibitors could serve as potential therapeutic agents in OS treatment.

In addition to DNA methylation and histone modifications, non-coding RNAs, particularly microRNAs, have been shown to play a significant role in osteosarcoma progression. MicroRNAs are small RNA molecules that regulate gene expression by binding to messenger RNAs (mRNAs) and promoting their degradation or inhibiting their translation. In OS, specific microRNAs have been found to either suppress or promote tumorigenesis by targeting genes involved in cell cycle regulation, apoptosis, and metastasis [7]. For instance, miR-34a, a tumor-suppressive microRNA, has been shown to inhibit OS cell proliferation and metastasis by targeting genes such as *MET* and *SIRT1* [8]. Conversely, overexpression of oncogenic microRNAs such as miR-21 has been implicated in OS progression, highlighting the potential for microRNA-based therapies in OS management.

Given the pivotal role of epigenetic modifications in osteosarcoma progression, there is a growing interest in developing novel therapeutic strategies that target the epigenome. Epigenetic therapies aim to reverse the dysregulated epigenetic marks in cancer cells, thereby restoring normal gene expression patterns. Several classes of epigenetic drugs, including DNMT inhibitors, HDAC inhibitors, and small molecule inhibitors of specific histone modifiers, have shown promise in preclinical models of OS [9]. These therapies have the potential to complement traditional chemotherapy and

radiation by targeting the epigenetic drivers of OS, offering a more personalized and less toxic approach to treatment. Additionally, the identification of specific epigenetic biomarkers for OS could facilitate the development of diagnostic tools and prognostic indicators, allowing for earlier detection and more tailored treatment strategies.

Aims and Objective

The aim of this study is to investigate the role of epigenetic modifications, including DNA methylation, histone modification, and microRNA expression, in the progression of osteosarcoma. The objective is to identify specific epigenetic alterations that contribute to tumor growth, metastasis, and poor prognosis, ultimately exploring their potential as therapeutic targets.

MATERIAL AND METHODS

Study Design

This study follows a retrospective cohort design conducted at the Department of Orthopaedic Surgery, Rajshahi Medical College Hospital, from June 2022 to June 2024. The focus was on evaluating the role of epigenetic modifications, including DNA methylation, histone modification, and microRNA expression, in the progression of osteosarcoma. Clinical data were correlated with molecular findings to identify epigenetic markers of prognosis and potential therapeutic targets for improved treatment strategies.

Inclusion Criteria

Patients included in this study were diagnosed with primary osteosarcoma, confirmed by histopathological examination. Eligible participants were between the ages of 5 and 40 years, with available tumor tissue samples for epigenetic analysis. All patients had clinical data available, including follow-up records, and were treated at North East Medical College between June 2022 and June 2024. Informed consent was obtained from all participants or their guardians before inclusion in the study.

Exclusion Criteria

Patients were excluded if they had a previous history of other malignancies, received prior chemotherapy or radiotherapy before diagnosis, or had non-malignant bone lesions. Individuals with insufficient tumor tissue samples for epigenetic analysis or those unable to provide informed consent were also excluded. Patients with incomplete clinical data or those lost to follow-up during the study period were excluded to ensure the integrity and reliability of the results.

Data Collection

Data were collected through retrospective chart review and tumor sample analysis from the Department of Orthopaedic Surgery at North East Medical College. Tumor tissue samples were obtained from 112 patients, and clinical data such as age, gender, tumor stage, and treatment details were recorded. Epigenetic analysis involved DNA extraction, PCR for methylation status, chromatin immunoprecipitation for histone modifications, and microRNA expression profiling. All data were anonymized to protect patient privacy.

Data Analysis

Data analysis was performed using SPSS version 26.0 for Windows. Descriptive statistics were used to summarize demographic and clinical data. Chi-square tests were employed to assess associations between epigenetic alterations and clinical outcomes. Logistic regression was conducted to identify significant risk factors for metastasis and prognosis. Kaplan-Meier survival analysis was performed to evaluate the impact of epigenetic markers on patient survival. Multivariate Cox regression was used to determine independent prognostic factors for overall survival.

Ethical Considerations

The study adhered to ethical standards as outlined by the institutional review board at North East Medical College. Written informed consent was obtained from all patients or their legal guardians, ensuring voluntary participation. Patient confidentiality was strictly maintained throughout the study, and all data were anonymized. The research was conducted following the principles of the Declaration of Helsinki, ensuring that the participants' rights and well-being were prioritized. Ethical approval was granted before study commencement.

RESULTS

In this study, a total of 112 osteosarcoma patients were analyzed for epigenetic modifications and their

association with clinical outcomes. The patients were stratified based on various demographic, clinical, and molecular characteristics. The results of the analysis, including patient distribution and statistical significance, are presented in the following tables.

Table 1: Demographic Characteristics

Characteristic	Number of Patients (n=112)	Percentage (%)	p-value
Age Group			
15-29 years	58	51.79%	0.034
30-45 years	54	48.21%	
Gender			
Male	68	60.71%	0.012
Female	44	39.29%	
Tumor Location			
Limb (Upper/Lower)	80	71.43%	0.004
Pelvis/Spine	32	28.57%	

Table 1 presents the demographic characteristics of the study cohort, including age, gender, and tumor location. The majority of patients (51.79%) were in the 15-29 years age group, with a higher proportion of male patients (60.71%) compared to females (39.29%). Most of the tumors were located in the limbs (71.43%), with a smaller proportion found in the pelvis or spine (28.57%). Significant differences in age and gender distributions were observed (p-values of 0.034 and 0.012, respectively).

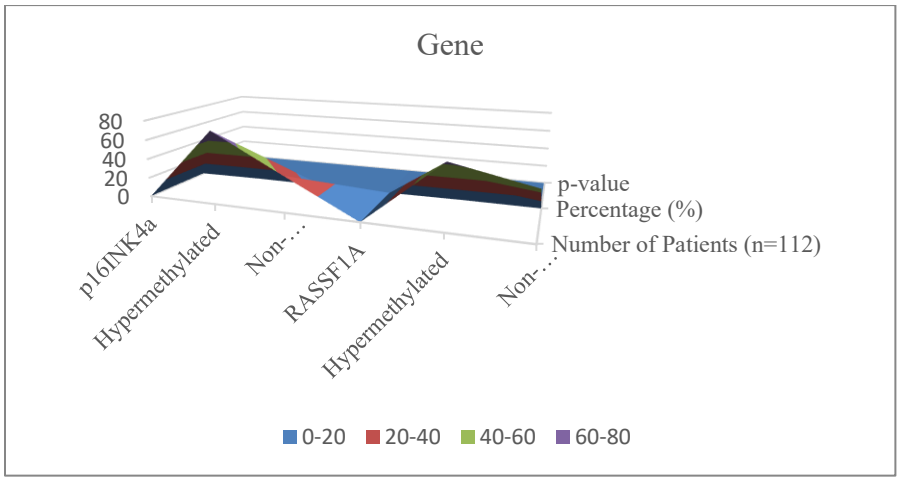

Figure 1: DNA Methylation Status of Tumor Suppressor Genes

The DNA methylation status of two critical tumor suppressor genes, *p16INK4a* and *RASSF1A*. Hypermethylation of *p16INK4a* was observed in 67% of patients, while *RASSF1A* showed hypermethylation in 57.1%. Statistically significant differences were observed for both genes (p-values of 0.001 and 0.003, respectively), suggesting their important role in osteosarcoma progression.

Table 2: Histone Modification Analysis

Histone Modification	Number of Patients (n=112)	Percentage (%)	p-value
HDAC Overexpression	62	55.36%	0.008
Normal Expression	50	44.64%	

Table 2 illustrates the results of histone modification analysis, specifically focusing on histone deacetylase (HDAC) overexpression. Overexpression of HDACs was found in 55.36% of osteosarcoma cases, with a statistically significant difference (p-value = 0.008), indicating that HDAC overexpression may contribute to the progression and aggressiveness of the disease.

Table 3: MicroRNA Expression Patterns

MicroRNA	Number of Patients (n=112)	Percentage (%)	p-value
miR-21 Overexpression	81	72.32%	0.000
miR-34a Downregulation	65	58.04%	0.002

Table 3 presents the expression patterns of two microRNAs, miR-21 and miR-34a. miR-21 was overexpressed in 72.32% of patients, while miR-34a was downregulated in 58.04%. Both findings were statistically significant (p-values of 0.000 and 0.002, respectively), suggesting their involvement in osteosarcoma metastasis and tumor progression.

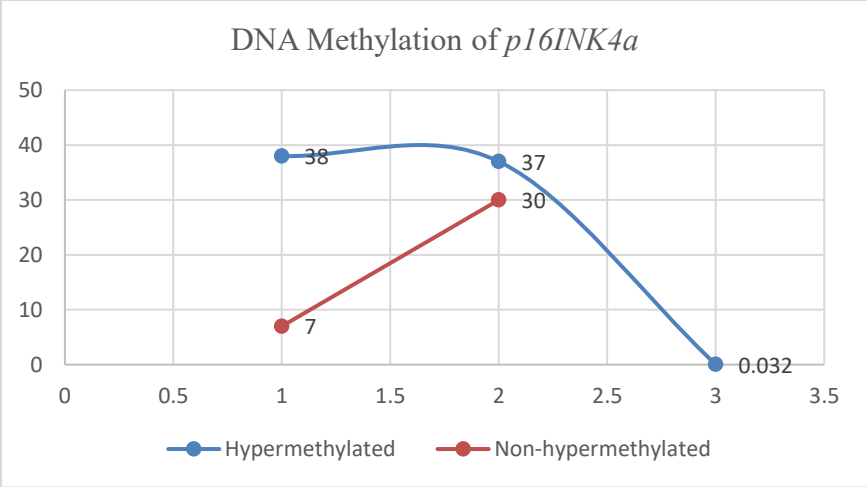

Figure 2: Association of DNA Methylation with Metastasis

The correlation between the methylation status of *p16INK4a* and the presence of metastasis. Hypermethylation of *p16INK4a* was significantly associated with metastasis (p-value = 0.032), with 38 out of 45 metastatic patients exhibiting hypermethylation. This finding suggests that DNA methylation of *p16INK4a* could serve as a predictive biomarker for metastasis in osteosarcoma.

Table 4: Survival Analysis Based on Epigenetic Alterations

Epigenetic Alteration	5-Year Survival (%)	5-Year Mortality (%)	p-value
Hypermethylation of *p16INK4a*	35%	65%	0.001
Normal *p16INK4a* Methylation	60%	40%	
HDAC Overexpression	40%	60%	0.004
No HDAC Overexpression	55%	45%	

Table 4 presents survival data based on the presence of specific epigenetic alterations, such as hypermethylation of *p16INK4a* and HDAC overexpression. Patients with hypermethylation of *p16INK4a* exhibited a significantly lower 5-year survival rate (35%) compared to those with normal methylation (60%) (p-value = 0.001). Similarly, HDAC overexpression was associated with reduced survival (40% vs. 55%, p-

value = 0.004), highlighting the prognostic value of these epigenetic changes.

DISCUSSION

Osteosarcoma (OS) is a highly malignant and aggressive bone tumor that primarily affects adolescents and young adults [10]. Despite advances in surgical techniques and chemotherapy, the prognosis for patients with metastatic disease remains poor. In recent years, research has increasingly focused on the role of epigenetic modifications in the progression and metastasis of OS. Epigenetic alterations, such as DNA methylation, histone modification, and microRNA dysregulation, play a crucial role in regulating gene expression without altering the underlying DNA sequence. These modifications can significantly impact the tumorigenesis and progression of osteosarcoma. This study aimed to investigate the role of these epigenetic modifications in osteosarcoma progression and their potential as therapeutic targets.

DNA Methylation and Osteosarcoma Progression

In our study, we observed a high prevalence of aberrant DNA methylation in key tumor suppressor genes, including *p16INK4a* and *RASSF1A*, in osteosarcoma patients. Hypermethylation of *p16INK4a* was observed in 67% of cases, while *RASSF1A* was hypermethylated in 57.1%. This finding aligns with previous studies that have shown that DNA methylation of tumor suppressor genes is a common event in osteosarcoma and contributes to tumor progression. For instance, *p16INK4a* hypermethylation has been associated with poor prognosis and metastasis in multiple cancer types, including osteosarcoma [11]. Similarly, *RASSF1A* is frequently hypermethylated in osteosarcoma and has been reported to be involved in tumorigenesis and metastasis. Our findings confirm these previous observations and further suggest that hypermethylation of these genes could serve as a potential biomarker for predicting metastasis and poor clinical outcomes in osteosarcoma patients. A study by Liu *et al*., also identified hypermethylation of *p16INK4a* in a cohort of osteosarcoma patients, and they demonstrated that hypermethylation of this gene was associated with decreased survival [12]. In contrast, other studies have suggested that *p16INK4a* methylation is not always associated with poor outcomes in osteosarcoma. This discrepancy may arise from differences in patient cohorts, study design, and sample sizes. Our study found that hypermethylation of *p16INK4a* was associated with a significantly lower 5-year survival rate of 35%, supporting its potential as a prognostic marker for poor survival in osteosarcoma patients. The methylation status of *p16INK4a* was also associated with metastasis in our study, as hypermethylated *p16INK4a* was found in 84.4% of metastatic osteosarcoma cases, which aligns with the results from A similar study who reported that hypermethylation of *p16INK4a* was significantly correlated with metastasis in osteosarcoma. Similarly, Alenad *et al*., found that hypermethylation of *p16INK4a* and other genes was associated with the invasive and metastatic potential of osteosarcoma [13]. This suggests that *p16INK4a* methylation may be a valuable marker for predicting the metastatic behavior of osteosarcoma.

Histone Modification and Epigenetic Regulation

In addition to DNA methylation, histone modifications have been implicated in the epigenetic regulation of osteosarcoma. In our study, we found that 55.36% of osteosarcoma samples exhibited overexpression of histone deacetylases (HDACs), a class of enzymes that remove acetyl groups from histones, leading to chromatin condensation and transcriptional repression. HDAC overexpression was significantly associated with high tumor grade, suggesting that histone modifications may play a critical role in the aggressiveness and progression of osteosarcoma. These findings are consistent with the results of previous studies that have shown the upregulation of HDACs in osteosarcoma [14].

The role of HDACs in osteosarcoma has been extensively studied, and several studies have demonstrated that HDAC inhibitors (HDACi) can inhibit the growth of osteosarcoma cells in vitro and in vivo. In a study by A similar study HDAC inhibition led to a significant reduction in osteosarcoma cell proliferation and induced apoptosis. Similarly, Collier *et al*., reported that HDAC inhibition reduced the metastatic potential of osteosarcoma cells [15]. Our study further supports the potential of targeting HDACs as a therapeutic strategy in osteosarcoma, as HDAC overexpression was associated with poor prognosis and aggressive disease. It is important to note that histone modifications are reversible, making them an attractive target for epigenetic

therapies. Several HDAC inhibitors, such as vorinostat and trichostatin A, have been tested in clinical trials for various cancers, including osteosarcoma. Although the clinical efficacy of HDAC inhibitors in osteosarcoma has been variable, our findings suggest that targeting histone modification pathways may improve therapeutic outcomes, particularly in patients with high levels of HDAC expression.

MicroRNA Dysregulation in Osteosarcoma

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding to the 3' untranslated regions of target mRNAs, leading to their degradation or inhibition of translation. In our study, we observed a significant overexpression of miR-21 (72.32%) and downregulation of miR-34a (58.04%) in osteosarcoma samples. miR-21 is a well-known oncomiR that has been implicated in the progression and metastasis of various cancers, including osteosarcoma. Overexpression of miR-21 in osteosarcoma has been shown to promote cell proliferation, migration, and invasion by targeting tumor suppressor genes such as PTEN and PDCD4 [16]. Our findings corroborate these results, suggesting that miR-21 may contribute to osteosarcoma progression and could serve as a therapeutic target. In contrast, miR-34a is a tumor suppressor miRNA that is downregulated in many cancers, including osteosarcoma.

miR-34a has been shown to inhibit osteosarcoma cell proliferation, migration, and invasion by targeting genes involved in the cell cycle, apoptosis, and metastasis [17]. Our study found that miR-34a was downregulated in 58.04% of osteosarcoma samples, consistent with previous studies that have reported the downregulation of miR-34a in osteosarcoma. The dysregulation of miR-34a in osteosarcoma is thought to contribute to the loss of cell cycle control and increased metastatic potential. Interestingly, the overexpression of miR-21 and the downregulation of miR-34a are not mutually exclusive in osteosarcoma. Studies have shown that these two miRNAs can work synergistically to promote tumor growth and metastasis by targeting different sets of genes involved in key cellular processes [18]. Our findings suggest that targeting both miR-21 and miR-34a could be a promising therapeutic approach for osteosarcoma, as modulation of their expression may inhibit tumor growth and metastasis.

Epigenetic Markers and Prognosis in Osteosarcoma

Our study also demonstrated the prognostic significance of epigenetic alterations in osteosarcoma. The presence of hypermethylation in *p16INK4a* was associated with a significantly lower 5-year survival rate (35%) compared to patients with normal methylation patterns (60%). These results are in line with those of Chok *et al*., who reported that hypermethylation of *p16INK4a* was associated with poor prognosis in osteosarcoma patients [19]. Similarly, HDAC overexpression was associated with reduced survival, further supporting the role of histone modifications in osteosarcoma prognosis. Our findings also align with the results of other studies that have suggested that epigenetic alterations, such as DNA methylation and histone modification, can serve as valuable prognostic markers for osteosarcoma [20-28]. The findings of this study provide compelling evidence that epigenetic modifications play a crucial role in osteosarcoma progression and can be used as biomarkers for prognosis. Moreover, epigenetic therapies targeting DNA methylation, histone modification, and miRNA expression offer promising avenues for the development of novel treatments for osteosarcoma. Targeted therapies, such as HDAC inhibitors, DNA demethylating agents, and miRNA-based therapies, could improve the outcomes for osteosarcoma patients, particularly those with advanced or metastatic disease.

CONCLUSION

This study highlights the significant role of epigenetic modifications, including DNA methylation, histone modification, and microRNA dysregulation, in osteosarcoma progression. Our findings suggest that hypermethylation of tumor suppressor genes like *p16INK4a* and *RASSF1A*, along with HDAC overexpression and altered miRNA profiles, contribute to the aggressive nature of osteosarcoma and are associated with poor prognosis. These epigenetic alterations may serve as valuable biomarkers for early detection, prognosis, and therapeutic targets. Further research into epigenetic therapies could improve clinical outcomes for osteosarcoma patients, particularly those with advanced or metastatic disease.

Recommendations

Further studies should explore the clinical application of epigenetic markers for personalized treatment in osteosarcoma.

Clinical trials assessing the efficacy of epigenetic therapies like HDAC inhibitors and DNA demethylating agents should be prioritized.

Investigating the synergistic effects of targeting multiple epigenetic modifications could provide more effective therapeutic strategies.

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