**Dear Editor,**

**Please find enclosed a modified version of my Microreview manuscript “MicroRNA: Small but Mighty Effects on Neuroblastoma”. To address the concerns and comments raised by the 3 reviewers, I made the following changes to improve and clarify the manuscript. I hope that these changes make the manuscript acceptable for publication in Microreviews in Cell and Molecular Biology.**

**Sincerely,**

**Kate Chandler**

**Reviewer 1: I agree with the comments of the reviewer, adding in-text citations and transition words suggested by this reviewer were very helpful and allowed me to improve the quality of my Microreview manuscript. The suggestion of adding intext citations made by reviewer 1 helped call attention to an aspect I missed and has been corrected by adding in-text citations.**

**Reviewer 2: I agree with the comments of the reviewer, adding in-text citations and fixing some grammar mistakes suggested by this reviewer were very helpful and allowed me to improve the quality of my Microreview manuscript. The suggestion of adding intext citations and fixing grammar mistakes made by reviewer 2 helped and has been corrected by adding in-text citations and fixing a few grammar mistakes.**

**Reviewer 3: I agree with the comments of the reviewer, adding in-text citations suggested by this reviewer was very helpful and allowed me to improve the quality of my Microreview manuscript. The suggestion of adding intext citations made by reviewer 3 helped call attention to an aspect I missed and has been corrected by adding in-text citations.**

**[MicroRNA: Small but Mighty Effects on Neuroblastoma]**

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**Neuroblastoma is the most common extracranial solid tumor occurring in childhood that also is associated with a poor prognosis due to resistance to chemotherapy and genetic amplification of *MYCN*. MicroRNA are non-coding RNAs that have a very important role in regulating the expression of genes and function as chemical messengers to mediate cell-cell communication. The role microRNA’s plays in drug-resistance development in the tumor microenvironment as well as the amplification of *MYCN* is currently being studied. More specifically the assessment of which exosomic miRNA’s are involved and which molecular mechanisms they elicit this function. Recent progress has been made through data showing that there are several important roles microRNA plays in NBL. One such role is the exosomic role of miRNA-21 and miRNA-155 in “talking” between monocyte and NBL cells that contribute to resistance to chemotherapy in a novel signaling pathway. Another role is how the loss of let-7, a tumor suppressor family of microRNA’s, is associated with amplification of *MYCN* in NBL that possibly also has a more broad implication in cancer pathogenesis. Questions remain in how these findings can be contributed to helping improve prognoses and implications into treatment.**

**Introduction**

Neuroblastoma (NBL) is the most common extracranial solid tumor that occurs almost exclusively in infants and children. Many cases of NBL are associated with poor prognosis and outcomes despite current standard treatments, this is due to developing resistance to treatments. (Kishore) MicroRNA (miRNA) were suspected to play a large role in drug-resistance development and amplification of tumor suppressor families in numerous cancers but were, until recently, largely unexplored. MiRNA is non-coding RNAs that have a very important role in regulating the expression of genes and function as chemical messengers to mediate cell-cell communication. The role of miRNA has been studied in many other cancers but not in NBL until recently. MiRNA is associated with the genetic amplification of *MYCN*. (Powers) *MYCN* is a gene that plays an important role in providing instructions for the creation of proteins for the formation of tissues and organs during development as well as regulating cell growth and apoptosis. (Powers)*MYCN* also helps regulate other gene activity by binding to specific regions of DNA and controlling transcription. (Powers) The *MYCN* gene amplification is associated with many of the poor prognoses in NBL. *MYCN* is also a target of a tumor suppressor family of microRNAs called *let-7*. Genetic loss of *let-7* is commonly inhibited by *LIN28B* and suspected to allow amplification of *MYCN* to contribute to NBL development as well as other cancers. (Powers) Exosomic miRNA are also suspected to play a role in chemotherapy resistance through their release within the tumor microenvironment (TME). Tumor-associated macrophages (TAMs) are suspected to affect the resistance of NBL to chemotherapy through the exchange of exosomic miRNAs. TAMs are heavily involved in cancer inflammation and promote the growth, metastasis, and development of drug resistance that contributes to poor prognosis in NBL.

 Another important role of microRNA in Neuroblastoma is its role in signaling pathways. Phosphatidylinositol-3-kinase (PI3K) signaling is a very important intracellular pathway and a very important regulator for cancer. PI3K is a part of the PI3K/AKT/mTOR signaling pathway. This pathway regulates cell growth, motility, survival, angiogenesis, and metabolism which all play large roles in the development of cancer. (Yang) PI3K pathway is also important in combating the drug-resistant capabilities of NBL. PI3K pathway is dysregulated in almost all cancers and miRNA are crucial regulators of gene expression.

**Recent Progress**

Until recently NBL and the role of miRNA were largely unstudied despite the fact that it had been researched in several other cancers. In the last 5 years, recent progress has been made in addressing what role microRNA’s play, more specifically in drug-resistance development in the tumor microenvironment as well as the amplification of *MYCN*. *LIN28B* sparked interest due to it being highly expressed in human NBL and how that expression correlates to the tumor stage. *LIN28B* has been the topic of a recent study and found to suppress *let-7* which in turn is unable to play its critical role in regulating *MYCN* and NBL cell growth. Although more than just *LIN28B* were studied to determine the different methods NBL uses to disrupt *let-7* to continue overexpression of *MYCN*.

The research of the effects of miRNA in chemotherapy resistance used coculturing of NBL cells with human monocytes for 48 hours to test the theory of cross-talk between the two in resistance to chemotherapy in a novel exosomic pathway. More specifically to assess which exosmic miRNA’s are involved and which molecular mechanisms they elicit this function. The coculturing tests performed were used to assess the exosomic transfers of miR-155 from human monocytes to NBL cells and miR-21 from NBL cells to human monocytes. These discoveries were found through studies on mice using Luciferase reporter assays to assess miR-155 targeting of TERF1 in NBL cells. Tumor growth was measured using NBL xenografts, and the levels of CD163, miR-155, and TERF1 were assessed using 20 NBL primary tissues by Human Exon Arrays. This accomplished a greater understanding of the role of MiRNA and which exosmic miRNA’s are involved and which molecular mechanisms they elicit these functions.

Recently, there has also been more advanced research reaching clinical trials in targeting the PI3K/AKT/mTOR signaling pathway. MiRNA is being researched to determine its ability to regulate this pathway and how it can be used in future advances for therapeutic uses. PI3K pathway has been found to be dysregulated in almost all cancers so the use of miRNA to help reverse this effect or stop it from happening would help prevent or stop the spread of cancer. The cause of initial dysregulation has been seen in various mechanisms such as inactivation of tumor suppressors and mutation of PI3K. Patients in these clinical trials have been given doses of reversible inhibitors of PI3K in hopes of regulating the pathway. The inhibitor has shown to be more effective in patients with the PI3K pathway but some without the mutation also benefited. Although its results have not shown promise for a wider implication of all cancers due to resistance. The PI3K pathways are very complex, involving many feedback loops, and extensive crosstalk nodes, and other signaling pathways. This makes it difficult to pinpoint where clinical trials have gone wrong from resistance. This resistance has made it difficult for many therapeutic drugs to gain much efficiency. Researchers are working to better understand this pathway and the effects of miRNA to better combat this resistance and provide better therapeutic responses.

**Discussion**

Their data found has found that there are several important roles microRNA plays in NBL. For example, one such role is the exosomic role of miRNA-21 and miRNA-155 in “talking” between monocyte and NBL cells that contribute to resistance to chemotherapy in a novel signaling pathway. The discovery of this novel pathway has helped open the door for new studies on how exactly signaling pathways can be used to affect the chemotherapy resistance in NBL. These findings prove to hold significance through their results but need further investigation the specific levels of concentration of exosomic miR-21 trigger TLR8-dependent response in TAMs. Also how other targets might be affected. Further studies would need to be done to move forward in chemotherapy resistance determination in NBL. Though until recently not many studies have been conducted on the effects of microRNA on NBL, the findings of miR-21 and miR-29a in lung cancer also help to provide an expectancy in NBL.

The research for *let-7* lead to the discovery that there is not one but several different mechanisms that NBL uses to target the *let-7* function. *LIN28B* was discovered to not be the only method that is used by NBL to disrupt the function of *let-7.* The research of *let-7* has exposed a new doorway to establishing *let-7* restoration in a new possible therapeutic goal to help target *MYCN* and also has a more broad implication in cancer pathogenesis. In spite of the fact that many attempts to target *MYCN* have been met with little success in the research of the last 20 years so its effects are still questionable until tested therapeutically. Although amplification of the *MYCN* gene only occurs in about 30% of tumors, the discovery of utilizing *let-7* to target *MYCN* would not be therapeutic to all tumors. In spite of the fact that this 30% defines a group of NBLs with a high risk of recurrence, continued research to better understand NBL is warranted. Questions still remain in how these findings can be contributed to helping improve prognoses and effectiveness in treatment.

Research of the PI3K pathway has to lead to many promising results even stated in one article “it is becoming increasingly clear that PI3K inhibitors are effective in inhibiting tumor progression.” (Yang) This as well as the approval of the first PI3Ki compound, PI3K delta-specific inhibitor idelalisib by the Food and Drug Administration (FDA) shows the need for further research into this pathway to be warranted. Although a recent study has shown that clinical trials with PI3K inhibitors “have shown limited clinical activity, possibly as a consequence of resistance to PI3K inhibition and poor tolerability of PI3K inhibitors.” The inhibitor has shown to be more effective in patients with the PI3K pathway but some without the mutation also benefited. This data suggests that more information is needed in order to maximize the ability of this inhibitor to provide a more efficient way to combat cancer. More research of the pathways would continue to clarify resistance mechanisms and help increase efficiency.

NBL research is continuing to reach new heights with research in miRNA effects within the body. These effects have large implications in cancer research and further studies continue to provide a greater understanding. A greater understanding not only for *LIN28B*, PI3K pathway, and other regulatory pathways but also for many other findings as well.

**References**

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