Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

MicroRNA-Induced Silencing of Glioma Progression

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Review of Kefas et al.

MicroRNAs (miRNAs) are a class of small, noncoding RNAs (~22–25 nt in length) that were originally discovered in *Caenorhabditis elegans* (Lagos-Quintana et al., 2003). Since this discovery, over ~4500 miRNAs have been identified in worms, plants, vertebrates, and viruses (Lagos-Quintana et al., 2003). miRNAs are encoded in introns and in clusters of noncoding regions in the genome, where their expression is tightly regulated in a cell-specific manner. Consequently, miRNA expression profile often varies between cell types. miRNAs are produced first by the cleavage of its primary transcript by the RNAase III endonuclease Drosha to release a 60–70 nt stem-loop intermediate known as the pre-miRNA. The pre-miRNA is then exported to the cytoplasm, where it is further cleaved by another RNase III endonuclease Dicer to produce a double-stranded RNA duplex consisting of the mature miRNA and its antisense strand. During the Dicer cleavage, the mature miRNA is coupled with the RNA-induced silencing complex (RISC), whose core components are the Argonaute family proteins (Ago1–4). The miRNA then directs the RISC to its target messenger RNA (mRNA), which it recognizes through partial sequence complementation at the 3′ untranslated region (UTR) of the mRNA transcript. The RISC complex associated with the miRNA would either silence protein translation or target the mRNA for degradation by Dicer. Overall, miRNAs are highly specific inhibitors of protein expression that are endogenous to specific cell types. Functional studies revealed that the miRNAs play an important role in regulating various cellular processes, including stem cell renewal and differentiation (DeSano and Xu, 2009). For example, miRNA-326 is commonly downregulated in hematopoietic precursors, and can stimulate differentiation of these cells by targeting and transcription factor Ets-1 (Du et al., 2009). Another example is the Let-7 family of microRNAs (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) that are predominantly downregulated in embryonic stem (ES) cells, and can drive differentiation of ES cells by targeting Notch (Wang et al., 2009). As Notch signaling is also involved in promoting stem cell renewal (Clark et al., 2007), Notch inhibition by Let-7 family of miRNAs also results in a decline in stem cell renewal. While the downregulation of these miRNAs would help maintain the "stemness" of stem cells or progenitors, their aberrant downregulation could also contribute to the development of tumors (DeSano and Xu, 2009).

A key feature of most malignant cancers is the presence of a small population of stem-like cancer cells, in which miRNAs such as miR-326 are downregulated (DeSano and Xu, 2009; Kefas et al., 2009). Stem-like cancer cells have been well characterized in gliomas, and are often referred to as glioma stem cells (GSCs) as they are capable of self-renewal and multilineage differentiation (Singh et al., 2004). GSCs are highly tumorigenic and invasive, with the potential of producing highly infiltrative tumors similar to most malignant gliomas (Singh et al., 2004). Therefore, GSCs are often considered the "bad seed" contributing to the malignancy of gliomas, and are currently the target for novel therapeutics (Singh et al., 2004). One therapeutic approach is to inhibit the expression of tumorigenic signaling pathways by specifically overexpressing the aberrantly downregulated miRNAs in GSCs. A candidate pathway contributing to glioma progression is the Notch signaling pathway. Notch is a transmembrane protein that is activated upon binding to its ligand Jagged-1 or Delta. Upon activation, Notch undergoes α-secretase and γ-secretase-dependent cleavage to liberate a notch intracellular domain (NICD), which translocates to the nucleus to drive the expression of Hes and Hey transcription factors. The downstream gene expression is crucial for Notch-mediated stem cell renewal, as well as maintenance of the "stemness" of stem cells or progenitors. Inhibition of Notch has been shown to inhibit the progression of gliomas, particularly the renewal and maintenance of GSCs (Clark et al., 2007). Notch is regulated by a number of miRNAs, including miR-1, miRNA-34, miRNA-146, miRNA-199, and Let-7 family of microRNAs, and aberrant downregulation of these miRNAs is often implicated in the development of various cancers, including gliomas (Wang et al., 2009). Therefore, these miRNAs are often selected as candidates for novel anti-cancer therapeutics.

Intriguingly, a recent article by Kefas et al. (2009) in *The Journal of Neuroscience*

Received Jan. 21, 2010; revised Feb. 11, 2010; accepted Feb. 16, 2010.
I thank Dr. William Jia for his advice and encouragement.
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DOI:10.1523/JNEUROSCI.0442-10.2010
 0270-6474/10/303868-02$15.00/0

has revealed a novel regulatory relationship between the neuronal miRNA-326 and Notch signaling in glioma cells. miRNA-326 is one of the miRNAs that are aberrantly downregulated in clinical gliomas. In this study, Kefas et al. (2009) found that in both glioma cells (GCs) and GSCs, Notch knockdown with small interfering RNA (siRNA) could stimulate upregulation of miRNA-326. On the other hand, when they transfected GC or GSCs with miRNA-326, they observed a decline in Notch signaling and subsequent reduction in the viability, proliferation, and invasiveness of GCs and GSCs *in vitro* [Kefas et al. (2009), their Fig. 4, Fig. 5]. They further found that miRNA-transfected GCs produced smaller tumors than control GCs [Kefas et al. (2009), their Fig. 4*F*]. Last, the effects of miRNA-326 expression could be reversed by upregulating Notch signaling [Kefas et al. (2009), their Fig. 6]. Collectively, they concluded that Notch signaling in glioma cells is tightly regulated by a Notch/miRNA-326 feedback loop, which they termed as the "Notch/miRNA-326 axis." Moreover, manipulations of this axis could influence the tumorigenic nature of GCs and GSCs.

The present study provided a fine example of the profound effects of miRNA dysregulation on GC and GSC behavior, and consequently glioma progression. In light of current data demonstrating differences between cancer and noncancer miRNA profiles, this article strongly supports that dysregulated miRNAs in gliomas could potentially be exploited in anti-glioma therapeutic. A unique feature of this study is its proof in principle that the dysregulated miRNA-326 could target the "bad seeds" in glioma, specifically by modulating Notch signaling in GSCs.

Although this study presented an elegant story, three caveats may need to be considered. First, the data presented in this study only rely on Notch siRNA knockdown to establish the regulatory relationship between Notch and miRNA-326. Mechanistically, the authors concluded that the Notch protein directly inhibits miRNA-326 expression. However, it is also possible that this effect could result from unknown interactions at an RNA level, perhaps between Notch mRNA and miRNA-326. Further studies to inhibit Notch at a protein level (using dominant-negative Notch or Notch inhibitors) may shed further light into this matter.

Another concern is that the authors have not discussed any differences between glioma cells and glioma stem cells, particularly in the context of the "Notch/miRNA-326 axis." It was assumed overall that Notch and miRNA-326 influence GCs and GSCs in a similar manner. Although this study is unique as it presented the first example that miRNA-326 could modulate the behavior of GSCs, it did not show whether miRNA-326 transfection would hinder the tumorigenicity of GSCs *in vivo*. Since GSCs are regarded as highly tumorigenic "seeds" involved in the invasive growth of gliomas, a question that arises is whether miRNA-326 transfection is equally effective in inhibiting the tumorigenic nature of glioma stem cells *in vivo*. As this fundamental question was not addressed, the efficacy of miRNA-326 in inhibiting the progression of glioma stem cell-derived tumors still remains speculative.

Last, the authors have not addressed how miRNA-326 could be delivered *in vivo*. A crucial question that arises is whether the delivery of miRNA-326 *in vivo* may influence Notch signaling not only in various nonstem and stem-like cells in the glioma tumor mass, but also in normal neural stem cells (NSCs). NSCs are actively produced in various germinal centers in the adult brain including the subventricular zone and dentate gyrus, where they are continually renewed in response to various signaling molecules including Notch. NSCs play a pivotal role in neurogenesis (generation of neurons), an active process regulating cognition and repair. It is unclear whether miRNA-326 would inhibit NSC survival and renewal in a manner similar to glioma stem cells, and whether that may result in side effects such as cognitive or neurological disorders. To use miRNA-326 as a therapeutic, as the authors suggested, it may be worth determining whether miRNA-326 can affect neural stem cells, and whether miRNA-326 could be delivered in a tumor-specific manner. Moreover, it may also be crucial to determine whether miRNA-326 would influence the translation of any other protein targets before application in an *in vivo* study.

Overall, this study presented miRNA-326 as a promising candidate to inhibit glioma progression. Clearly, further investigation into the role of miRNA-326/Notch axis in glioma stem cell signaling may better support the innovative use of miRNA-326 to target glioma stem cells, the "bad seed" in glioma progression.

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