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Brief Report

Towards the identification of miRNAs targeting the translational machinery as novel cancer therapeutics

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Abstract

Increased protein production is a prerequisite for cell proliferation, thus rendering translation and ribosome deregulation a common hallmark of cancer cell biology. A frequently observed mechanism in malignancies is the overactivation of the translational process. As a consequence, strategies that selectively target the ribosomal machinery bear significant promise as cancer therapeutic approaches. To this end, this report provides a workflow for the identification of novel miRNA molecules with the ability to target and suppress ribosomal activity in cancer cells.

Keywords: Cancer, miRNA therapeutics, Bioinformatics, Ribosome, Pharmacology

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Abbreviations:

RPs: Ribosomal Proteins

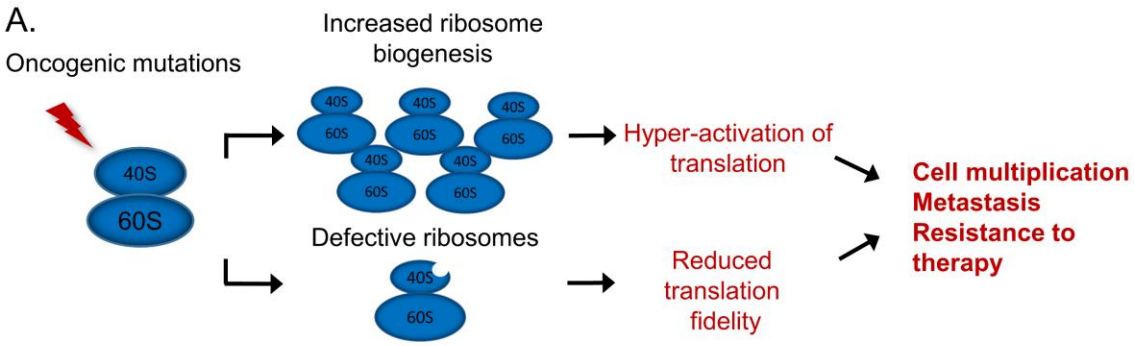
rRNAs: Ribosomal RNAs

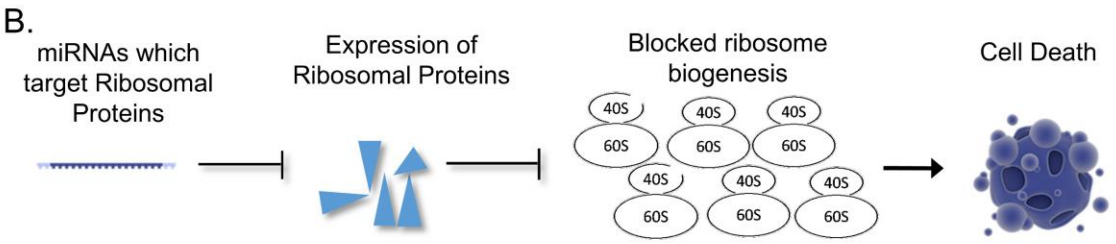
miRNA: MicroRNA

Ribosomes are the main molecular machines responsible for protein synthesis within the cells of all domains of life. Each ribosome is composed of 80 Ribosomal Proteins (RPs) and 4 Ribosomal RNAs (rRNAs) which assemble into two subunits within the nucleolus. After their export to the cytoplasm, the 40S and 60S subunits work together to convert genetic information into protein with both speed and accuracy.

Cancer cells reside in a permanent and uncontrolled cellular proliferation state. This trait induces a pressing need for protein production, which is crucial in order to support the incremented metabolic needs of the rapidly duplicating cells (Sulima et al., 2017). As a consequence, ribosomal function is often modulated in cancer cells with alterations mainly directing towards one or both of the following pathways: 1) to increase the abundance of the ribosomal machinery (and therefore protein synthesis); and/or 2) to reduce the translational accuracy (fig. 1A) (Topisirovic and Sonenberg, 2015, Tahmasebi et al., 2018, Robichaud et al., 2018). A notable example of the first mechanism is mediated via the oncogene MYC. The latter, is frequently amplified in various human cancers, where it has been shown to promote oncogenesis by acting as a direct transcriptional inducer of RPs genes (Robichaud et al., 2018). Alike MYC, additional factors, such as ΔN-netrin-1 and the Epithelial Cell Transforming 2 (ECT2) oncogene, attain aberrant functionality within tumor cells and function as strong inducers of rRNAs, thus driving ribosome biogenesis and vastly increasing protein synthesis (Delloye-Bourgeois et al., 2012, Huh et al., 2003). Interestingly though, lesions such as mutations, deletions or downregulation of specific RPs have, also, been observed in various types of cancers and those, although not always clearly understood, are presumed to impair accuracy of translation (second mechanism, fig. 1A). Examples include specific mutations in ribosomal proteins, such as uL18/RPL5 which was found mutated in patients' samples of glioblastoma (11%), melanoma (28%) and breast cancer (34%) (Fancello et al., 2017). Similarly, patients with acute lymphocytic leukemia repeatedly bear mutations in uL18/RPL5 and uL16/RPL10 (De Keersmaecker et al., 2013), whereas uS10/RPS20 mutations seem to play a role in the pathogenesis of a hereditary type of colorectal cancer (Nieminen et al., 2014). In summary, the entirety of the changes described above impair the central process of translation, thus inducing chaotic alterations in the cancer cell proteome, and ostensibly promoting cell multiplication, metastasis and resistance to therapy (fig. 1A).

In this direction, targeting the aberrant and uncontrolled ribosomal machinery of the cancer cell may constitute an effective anti-cancer strategy (outlined in fig. 1B). To this end, we have developed a strategy and a workflow pipeline to identify miRNAs with the ability to bind to RPs, and therefore leverage the gene-suppressing nature of the interaction between miRNAs and their target genes. Our goal is to unravel novel molecules of pharmacological interest aiming to selectively suppress the impaired-frequently overactivated- translation process of the tumor cells and thereby render those cells vulnerable to cell death (fig. 1B). Notably, the versatile class of miRNAs may include very potent pharmacological candidates as evidently shown by the multiple clinical trials that investigate such therapies as well as the recent approval





C.

miRNA name	No of RPs targeted (out of 80)
miR-484	30
miR-186-5p	29
miR-129-5p	12
miR-615-3p	26
miR-92a-3p	30
miR-16-5p	32
miR-100-5p	20
miR-320a	24
miR-92a-3p	30
miR-663a	12

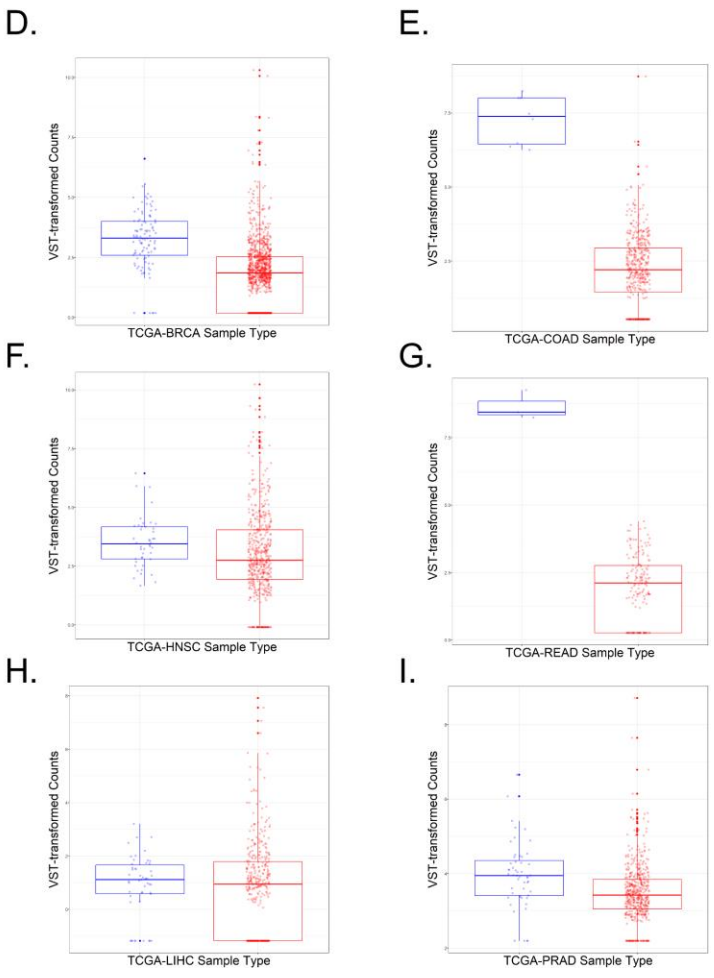


Figure 1: Outline of the main aspects addressed in order to identify miRNAs with the ability to target the ribosomal machinery.

A. Alterations in ribosomal function and oncogenesis. Oncogenic mutations affect ribosomal function via two main mechanisms: a) an increase in ribosome biogenesis (e.g. via the MYC

oncogene); and b) a reduction in translational fidelity (e.g. mutations in uL18/RPL5). Both mechanisms benefit cancer initiation and progression.

B. Targeting the ribosomal machinery with miRNA molecules. The suggested approach includes the identification of miRNAs that target RPs, thus suppressing ribosomal function in cancer cells and inducing cell death.

C. List of the miRNAs identified and number of RPs, out of the 80 expressed by the human genome, which are targeted by each miRNA. The results shown were generated using the online platforms miRWalk (Dweep et al., 2014) and miRNet (Fan et al., 2016).

D-I. miR-129-5p expression in patient samples from 6 cancer types. D. breast cancer, E. colon cancer, F. head & neck cancer, G. rectum cancer, H. hepatocellular cancer, I. prostate cancer. The gene expression data was retrieved from The Cancer Genome Atlas (TCGA) database. Blue boxes denote normal tissue, whereas red refers to tumor sample of the indicated cancer type.

of the first RNAi therapy. Prominent examples of such onco-suppressor miRNAs include miR-34a, let-7 and miR-16-5p, shown to inhibit the oncogene RAS (Yamakuchi et al., 2008), activate the onco-suppressor p53 (Cho, 2007), and block oncogenic signaling via VEGFA (Qu et al., 2017), respectively.

To identify our candidate miRNAs, we undertook an in-silico approach with the aim to analyze all the potential interactions between the known miRNAs (as annotated in miRBase) and the 80 RPs expressed by the human genome. The available databases that contain miRNA-mRNA interactions incorporate either predicted (usually generated by an algorithm) or validated interactions (generated during wet-lab experiments). For a thorough analysis we opted to include data from both types of datasets, using two platforms: miRNet for validated interactions (Fan et al., 2016) and miRwalk for predicted interactions (Dweep et al., 2014). Surprisingly, we identified a small group of 10 miRNAs whose members are reported to target at least 12 ribosomal proteins. Notably, four of those miRNAs seem to possess the ability to bind to more than 30 RPs encoding genes. This data suggests that the action of only a few miRNAs is adequate to modulate the expression of most RPs and thereby affect translation. Those miRNAs may represent potential biomolecules with anti-cancer activity. Such onco-suppressor miRNAs are frequently downregulated or deleted in cancer cells, thus removing this barrier in tumor initiation and progression. To investigate whether our miRNAs are subjected to this kind of regulation, we performed an expression analysis of miR-129-5p in patient samples from 6 cancers types (breast cancer, colon cancer, head & neck cancer, rectum cancer, hepatocellular cancer, and prostate cancer) (Fig1. D-I) by using the gene expression data deposited in The Cancer Genome Atlas (TCGA) database (Weinstein et al., 2013). Interestingly, we found that one of our candidate miRNAs, miR-129-5p is downregulated in all tested samples (Fig.1 D.). This is consistent with a hypothetical tumor-suppressor function of this miRNA and, likely, the anti-cancer activity of miR-129-5p is mediated via targeting ribosome biogenesis. Conclusively, this approach has resulted in the identification of a group of miRNAs

with potential modulatory role in ribosome biogenesis and at least one miRNA that seems to possess the capacity to act, upon its pharmacological exploitation, as anti-cancer therapeutic.

Treating cancer remains a pressing medical problem worldwide. By integrating biological knowledge as well as bioinformatic analysis we can not only identify potential ‘’druggable’’ targets but also discover pharmacological agents to block those targets. In this direction, our approach stems from the thorough understanding of ribosomal (de)regulation in cancer cells and integrates bioinformatic tools to identify miRNA molecules able to reverse the impaired translational activity of cancer cells. Indeed, our analysis suggests that at least 10 miRNAs may have a potential modulatory role in ribosomal function. We hypothesized that some of those miRNAs will act as tumor suppressors and, as a proof of concept we analyzed the expression of miR-129-5p across 6 cancer types. MiR-129-5p is consistently downregulated in cancer patients, suggesting that the miRNA exhibits potential anti-cancer activity. Thus, the delivery of miR-129-5p in patients will likely be associated with a beneficial therapeutic outcome and thereby, its study in cancer cell samples is highly interesting. However, a more thorough molecular analysis has to be carried out to pharmacologically exploit the possibility of successfully developing miRNAs as targeted anticancer therapeutics with increased efficacy and safety profiles in the clinical setting. In conclusion, this brief report provides a workflow for the identification of novel miRNA molecules with highly interesting properties in modulating ribosome function in cancer cells.

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