MICRO RNAs: THE TINY BLACK BOX IN DIAGNOSTICS!

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ABSTRACT:

The discovery of micro RNAs (miRNA) in cancer has opened up new opportunities in cancer research in recent years. Micro RNAs, that are small, endogenous, highly conserved, non-coding RNAs are now known to play crucial role in regulation of gene expression. They control genes associated with vital cellular processes like inflammation, cell cycle regulation, stress response, differentiation, apoptosis, and migration. In view of their important role in various key processes and pathways during tumour formation, as well as advancement, they are thought to be emerging diagnostic and prognostic biomarkers for different types of cancer. This review summarizes different pathways that lead to miRNA biogenesis and how they regulate gene expression.

KEYWORDS: micro RNAs, biogenesis, regulation of gene expression, cancer

INTRODUCTION:

About 70-80 % of human genome is transcribed into RNA, yet, only about 2% constitutes the protein-coding genes. For several years, this ‘so-called’ ‘junk DNA’ was believed to consist of non-coding genes forming non-coding RNA (ncRNA), not involved in any molecular or physiological processes. However, recent advances in high throughput sequencing and computational prediction methods have allowed the discovery and classification of several of these ncRNAs [PIWI-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), endogenous small interfering RNAs (endo-siRNAs or esiRNAs), microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), promoter associated RNAs (pRNAs)], some of which are speculated to have regulatory functions.

Since the discovery of Lin-4 miRNA in 1993, there had been numerous assumptions on the role of one of these non-coding RNAs, including the ‘miRNA’ in the modulation of gene expression. It has been evident from multiple studies that the expression of about 60% of the protein-coding genes is controlled by miRNA through mRNA degradation and/or translational suppression. MicroRNAs (miRNAs) are, therefore, post-transcriptional regulators of their mRNA targets. Although the majority of previous research have indicated that miRNAs have cytoplasmic functions, it is becoming obvious that miRNAs also have nuclear functions. Despite the fact that much research has gone into these areas, the method by which miRNAs select their mRNA targets and regulate downstream effector functions is still debatable.

1. WHAT IS miRNA?

miRNA is a non-coding single-stranded RNA, ~12-22 nucleotides long, that regulates gene expression by partnering with the mRNAs of protein-coding genes. These are transcribed from DNA through multiple intermediary components, including primary miRNA (pri-mRNA), precursor miRNAs (pre-mRNA), and mature miRNAs. Mature miRNAs interact with target mRNAs' 3'UTRs, which are helped by argonaut proteins, to regulate their synthesis. It should, however, be noted that, interactions of miRNAs with gene promoters, 5'UTRs or coding sequences have also been described and may result in distinct outcomes; for example, activation of gene expression instead of repression (Correia De Sousa et al., 2019). The degree of complementarity between mRNA and miRNA dictates// the final outcome- mRNA degradation, translational repression, or both. Many cellular processes, including proliferation, differentiation, apoptosis, innate and adaptive immune responses (Rodriguez A et al., 2007; O'Connell RM, 2007; Thai TH, 2007), metabolism (Poy MN et al., 2004, Esau C et al., 2006, and Martin MM., 2006), and development, are meticulously controlled by miRNAs (Ambros V, 2004). In view of the role played by miRNAs in gene expression, these tiny structures have been rightly called as the 'master regulators of gene expression' (Pedroza-Torres et al., 2019).

Approximately three percent of human genome codes for about 2,000 miRNA sequences, which regulate nearly thirty percent of human genes. miRNAs are important regulators of genes that play crucial roles in fundamental biological processes such as proliferation, differentiation and survival. They are important players in immune response, neural development, DNA repair, apoptosis, oxidative stress response and cancer. miRNAs influence the post-transcriptional regulation of important chromatin- and DNA-modifying enzymes, thus playing a vital role in the control of chromatin structure and gene expression. Each miRNA can regulate a variety of different genes (usually around 500), while each gene might be regulated by many different miRNAs, thus depicting a highly complex system (Favier et al., 2021) with alteration in miRNAs linked to deregulation of multiple key signalling pathways (Ye et al., 2019).

2. BIOGENESIS OF miRNAS:

Most often, miRNAs are synthesised by transcription of intronic, exonic, intergenic or mirtronic (within the introns of the mRNAs) loci in the genome, typically, but not always, by RNA Polymerase II (Lee et al., 2004, Pedroza-Torres et al., 2019). Biogenesis of the miRNA can occur via three pathways; canonical pathway, non-canonical pathway and Dicer-independent pathway.

2.1 Canonical pathway: Canonical pathway is the most predominant pathway for biogenesis of miRNA. Most miRNAs go through two maturation steps. The first occurs in the nucleus with the help of Drosha and DGCR8 and the second in the cytoplasm via RNAse III Dicer along with TARBP2.

Briefly, the miRNA genes transcribe to form the long pri-miRNA. The microprocessor complex [consisting of nuclear RNase Type III - Drosha and ds RNA binding protein-DiGeorge Syndrome Critical Region 8 (DGCR8)] further processes these pri-miRNAs. DGCR8 recognizes an N6-methyladenylated GGAC and other motifs within the pri-miRNA, whereas Drosha cleaves the pri-miRNA duplex. Thus, via the action of Drosha and DGCR8, the microprocessor complex produces a 70nt RNA molecule. This molecule has a two-nucleotide overhang at the 3'end called the precursor miRNA (pre-miRNA), which undergoes capping and polyadenylation to form a stem-loop structure.

The nuclear export factor-Ran/GTP/Exportin5 complex then transports the pre-miRNA from the nucleus to the cytoplasm. The 3' overhand is recognized by another protein called Dicer, an RNase III enzyme after it reaches the cytoplasm. This, in combination with transactivation-responsive RNA-binding protein 2 (TARBP2), results in the elimination of the terminal loop, resulting in a 22-nt mature dsRNA with a 2-nt overhang on both ends. In an ATP-dependent way, both ends can be presented to a large protein complex termed RISC, which contains the Argonaute (AGO) family of proteins. The stability at the 5' end determines which strand is loaded onto the AGO protein. Usually, the strand with the lowest stability is placed onto the AGO protein. The other strand is called the passenger strand and is unwound from the guide strand and cleaved by different cellular machinery. Thus, without the coordinated functions of Drosha, DGCR8, and Dicer, cells cannot produce canonical miRNAs.

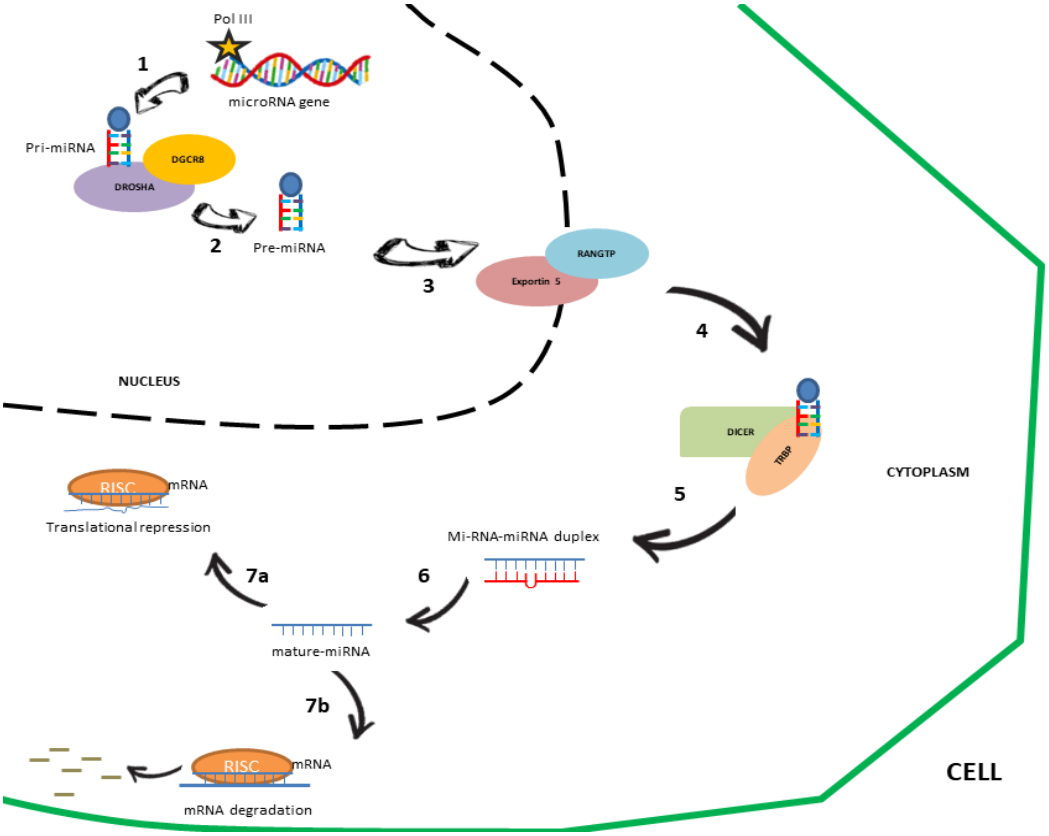

Fig. 1: The Canonical pathway

2.2 Non- canonical pathway:

In humans, approximately 60% of the interactions between the miRISC complex and mRNA are not entirely complementary and the regulation of expression occurs by non-canonical pathway (Condrat et al., 2020). Sequencing methods have shown that the non-canonical miRNAs are a subset of miRNAs that do not follow the canonical pathway and are produced by bypassing the microprocessor step in the classical canonical pathway. Dicer is almost always indispensable in the production of miRNAs by both, canonical and non-canonical pathways. However, Drosha and DGCR8 are only required to process canonical miRNAs. This alternative Drosha and DGCR8 independent pathway involves short intronic hairpins termed mirtrons that are spliced by spliceosome instead of cleavage by microprocessor to form an intermediate, which subsequently refolds into the pre-miRNA hairpins. This hairpin structure is directly suitable for dicer cleavage. Thus, this pathway bypasses the microprocessor complex by replacing it with splicing activity. Further, Exportin 5, a Ran-GTP dependent transporter, transports the pre-miRNA to the cytoplasm. This is followed by the coequal ensuing processes as the canonical pathway.

2.3 Dicer- independent pathway:

Unlike the canonical and non-canonical pathways outlined above, some miRNAs, such as miR-451 seen in humans, mice, and zebrafish, are synthesized via dicer- independent

pathway (Cheloufi S et al., 2010; Cifuentes D et al., 2010). In this process, the Pri-miR-451 is cleaved in the nucleus by Drosha/DGCR8 complex and a 18nt long pre-miRNA is synthesized. The pre-miRNA produced is very short and cannot be processed by Dicer. It is thus either loaded directly onto Ago1 or Ago2 after cleavage at the 3'end by an unknown endonuclease. Later it integrates with the RICS complex.

3. THE miRNA REGULOME:

The whole set of regulatory elements that regulate miRNA expression or are under control of miRNAs constitute the miRNA regulome (Hrovatin and Kunej, 2018). All these regulatory elements have a direct effect on miRNA expression, and in turn, multiple cellular effects.

miRNA-associated variations could be studied at various omics levels: genomics/DNA; transcriptomics/RNA; proteomics and epigenomics, all of which are crucial in determining miRNA levels as well as action/s.

Mutations in any/ all of the following would lead to altered miRNA expression profiles: overexpressed/ under expressed/ silenced/ differentially expressed miRNAs.

Alterations at genetic level: Mutations and polymorphisms in

- miRNA regulatory regions
- miRNA genes: miRNA seed regions, miRNA genes affecting processing by silencing machinery
- miRtrons
- miRNA processing machinery: DICER/ DROSHA/AGO family protein- can importantly reduce expression of tumour suppressing miRNAs, such as MIRLET7 family- are common in various tumours
- signalling proteins- miRNA processing proteins, namely, DICER, DGCR8 and XPO-5

Different types of mutations have thus been reported for miRNA genes leading to polymorphisms in miRNAs (present within miRNA's upstream regions, or resulting from variations in downstream targets and genes encoding for silencing machinery), SNPs and copy number variants (CNVs).

Epigenetic modifications of miRNA genes leading to varying patterns of expressions is another area of interest, as miR epimutations are reversible, raising the possibility of having therapeutics based on it (Thirlwell et al. 2011; Hrovatin and Kunej, 2018).

Apart from alterations at genetic level, there exist mechanisms that affect miRNA

processing and maturation, including, asymmetric miRNA strand selection and RNA editing- introduction of single nucleotide changes in primary miRNAs. Nucleotide modifications introduced by 'RNA editing' greatly impact the stability, maturation and activity of the miRNa by changing its specificity towards target mRNAs. This phenomenon has been shown to be highly significant in context of cellular stresses; e.g., hypoxia or endoplasmic reticulum (ER) stress as well as several cancers (De Sousa et al., 2019).

4. REGULATION OF GENE EXPRESSION BY MATURE miRNAS:

How mature miRNAs regulate gene expression further depends on multiple factors. The strand of the miRNA (5p or 3p), which is degraded (passenger strand) or incorporated in the RISC complex (guide strand) in turn decides the set of target mRNAs. Complementarity between the miRNA response elements (MRE) on the mRNA and the seed sequence on the miRNA strand determine the specificity of the RISC complex's action as well as decide whether the mRNA is degraded or whether its translation is blocked. (De Sousa et al., 2019).

miRs influence various key processes and pathways during tumour formation, as well as advancement. Regulation of expression of glucose transporters, enzymes involved in glycolysis, lactate metabolism, glutamine metabolism and oxidative phosphorylation, to name a few. miRNAs, being important mediators of these events in this 'metabolic reprogramming' process, a crucial transformative force for the cell, and a hallmark of cancer cells (Pedroza-Torres et al, 2019), it's quite logical to think of them as possible candidates to track the onset and progression of the disease!

5. MICRO RNAS IN DISESES DIAGNOSIS:

Though miRNAs are largely found intracellularly, a significant number of miRNAs have also been reported to be present outside the cells, especially various body fluids. These represent the 'circulating' miRNAs acting as 'extracellular communication RNAs' involved in 'cross-talk' between diseased cells and their microenvironment (Jayashree P., et al., 2024).

, circulating microRNAs had emerged as promising diagnostic and prognostic biomarkers for the noninvasive detection of diseases with high specificity and sensitivity. More importantly, specific microRNA expression signatures reflect not only the existence of early-stage diseases but also the dynamic development of advanced-stage diseases, disease prognosis prediction, and drug resistance. To date, an increasing number of potential miRNA biomarkers have been reported, but their practical application prospects are still unclear.

Various techniques like Northern Hybridization, Quantitative Reverse Transcriptase PCR, Digital PCR, Microarrays and Next generation sequencing are currently used for detection as well as quantification of miRNAs

TABLE 1: DIFFERENTIALLY EXPRESSED MIRNAS IN VARIOUS DISEASES:

Disease	Differentially expressed miRNAs	Reference
heart and brain tissues in Down syndrome	miR-802, miR-155, miR-125b-2, let-7c and miR-99a	Kuhn et al., 2008
Alzheimer's disease	miR-31, miR-93, miR-143, and miR146a	Dong et al., 2015
Parkinson's disease	miR-141, miR-214, miR-146b-5p, and miR-193a-3p	Dong et al., 2016
autism spectrum disorder	miR-483-3p and miR-1202	Vaccaro et al., 2018
Breast cancer	miR-210, miR-224, miR-109, miR-335, miR-140, miR-542	Jayashree P., et al., 2024
Liver cancer	miR-18, miR-21 miR-106, miR-182, miR-221/222, miR-224, miR-99, miR-195, miR-199, miR-326	Jayashree P., et al., 2024
Kidney cancer	miR-100, miR-378, miR-194, miR-204, miR-106, miR-21, miR-210 miR-155, miR-1233, miR-185, miR- 489, miR- 630	Jayashree P., et al., 2024
Pancreatic cancer	miR-21, miR-106, miR-155, miR-185, miR-191, miR-210, miR-212, miR-221, miR-222, miR-301a, miR-4465, miR-616	Jayashree P., et al., 2024
Colorectal cancer	miR-21, miR-210, miR-30, miR-320, miR-148, miR-320, miR-138, miR-590	Jayashree P., et al., 2024
Cervical cancer	miR-630, miR-21, miR-152, miR-155, miR-210, miR-9-5p, miR-200, miR- miR-106a, miR-17, miR-19d, miR-526b, miR-20	Jayashree P., et al., 2024
Cardiac hypertrophy	miR-23a, miR-23b, miR-24, miR-195, miR-199a, and miR-214	Ardekani, A. M., et al.,2010
Psoriasis	mir-203	Ardekani, A. M., et al.,2010
Systemic lupus erythematosus	miR-189, miR-61, miR-78, miR-21, miR-142-3p, miR 342, miR-299-3p, miR-198 and miR-298 miR-196a, miR-17-5p, miR- 409-3p, miR-141, miR-383, miR- 112, and miR-184	Ardekani, A. M., et al.,2010

6. CONCLUSION

Discovery of noncoding RNAs has opened new avenues in our understanding of regulation of gene expression. The situation becomes quite complicated, as miRNAs display both, pleiotropism as well as redundancy. A single miRNA can regulate multiple genes, and

multiple miRNAs may have effect on a single gene. Micro RNA profiles also change in various diseases including cancer at all stages viz., proliferation, metastasis, angiogenesis and apoptosis. The loss- and/ or gain-of-function of certain miRNAs appears to be a vital event in different stages various cancers. Micro RNAs would play promising role in diagnostics prognostics and therapeutics in near future.

REFRENCES:

1. Ardekani, A. M., & Naeini, M. M. (2010). The role of microRNAs in human diseases. *Avicenna journal of medical biotechnology*, *2*(4), 161.Ambros V. (2004). The functions of animal microRNAs. Nature, 431(7006), 350–355. https://doi.org/10.1038/nature02871

2. Cheloufi, S., Dos Santos, C. O., Chong, M. M., & Hannon, G. J. (2010). A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature, 465(7298), 584–589. https://doi.org/10.1038/nature09092

3. Cifuentes, D., Xue, H., Taylor, D. W., Patnode, H., Mishima, Y., Cheloufi, S., Ma, E., Mane, S., Hannon, G. J., Lawson, N. D., Wolfe, S. A., & Giraldez, A. J. (2010). A novel miRNA processing pathway independent of Dicer requires Argonaute2 catalytic activity. Science (New York, N.Y.), 328(5986), 1694–1698. https://doi.org/10.1126/science.1190809

4. Condrat, C. E., Thompson, D. C., Barbu, M. G., Bugnar, O. L., Boboc, A., Cretoiu, D., Suciu, N., Cretoiu, S. M., & Voinea, S. C. (2020). miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells, 9(2), 276. https://doi.org/10.3390/cells9020276

5. Correia de Sousa, M., Gjorgjieva, M., Dolicka, D., Sobolewski, C., & Foti, M. (2019). Deciphering miRNAs' Action through miRNA Editing. International journal of molecular sciences, 20(24), 6249. https://doi.org/10.3390/ijms20246249

6. Dong, H., Wang, C., Lu, S., Yu, C., Huang, L., Feng, W., Xu H., Chen, X., Zen, K., Yan, Q. Liu, W. & Zhang, C. Y. (2016). A panel of four decreased serum microRNAs as a novel biomarker for early Parkinson's disease. *Biomarkers*, *21*(2), 129-137.

7. Dong, H., Li, J., Huang, L., Chen, X., Li, D., Wang, T., Hu, C., Xu, J., Zhang, C., Zen, K., Xiao, S., Yan, Q., Wang, C. & Zhang, C. Y. (2015). Serum microRNA profiles serve as novel biomarkers for the diagnosis of Alzheimer's disease. *Disease markers*, *2015*(1), 625659.

8. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF,

Bhanot S, Monia BP. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab. 2006;3:87–98

9. Favier, A., Rocher, G., Larsen, A. K., Delangle, R., Uzan, C., Sabbah, M., Castela, M., Duval, A., Mehats, C., & Canlorbe, G. (2021). MicroRNA as Epigenetic Modifiers in Endometrial Cancer: A Systematic Review. Cancers, 13(5), 1137. https://doi.org/10.3390/cancers13051137

10. Hrovatin, K., & Kunej, T. (2018). Classification of miRNA-related sequence variations. Epigenomics, 10(4), 463–481. https://doi.org/10.2217/epi-2017-0126

11. Jayashree, P., Kalpita, M., Judith, T., Singh, A. S., & Ashwin, K. (2024). Role of MicroRNA in Hypoxic Tumours and their Potential as Biomarkers for Early Detection of Cancer. *Current Molecular Medicine*, *24*(5), 525-536.

12. Kuhn, D. E., Nuovo, G. J., Martin, M. M., Malana, G. E., Pleister, A. P., Jiang, J., Schmittgen , T. D., Terry, A.V., Gardiner, K., Head, E., Feldman, D., & Elton, T. S. (2008). Human chromosome 21-derived miRNAs are overexpressed in down syndrome brains and hearts.

13. Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H., & Kim, V. N. (2004). MicroRNA genes are transcribed by RNA polymerase II. The EMBO journal, 23(20), 4051–4060. https://doi.org/10.1038/sj.emboj.7600385

14. Martin, M. M., Lee, E. J., Buckenberger, J. A., Schmittgen, T. D., & Elton, T. S. (2006). MicroRNA-155 regulates human angiotensin II type 1 receptor expression in fibroblasts. The Journal of biological chemistry, 281(27), 18277–18284. https://doi.org/10.1074/jbc.M601496200 (Retraction published J Biol Chem. 2013 Feb 8;288(6):4226)

15. O'Connell, R. M., Taganov, K. D., Boldin, M. P., Cheng, G., & Baltimore, D. (2007). MicroRNA-155 is induced during the macrophage inflammatory response. Proceedings of the National Academy of Sciences of the United States of America, 104(5), 1604–1609. https://doi.org/10.1073/pnas.0610731104

16. Pedroza-Torres, A., Romero-Córdoba, S. L., Justo-Garrido, M., Salido-Guadarrama, I., Rodríguez-Bautista, R., Montaño, S., Muñiz-Mendoza, R., Arriaga-Canon, C., Fragoso-Ontiveros, V., Álvarez-Gómez, R. M., Hernández, G., & Herrera, L. A. (2019). MicroRNAs in Tumor Cell Metabolism: Roles and Therapeutic Opportunities. Frontiers in oncology, 9, 1404. https://doi.org/10.3389/fonc.2019.01404

17. Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P., & Stoffel, M. (2004). A

pancreatic islet-specific microRNA regulates insulin secretion. Nature, 432(7014), 226–230. https://doi.org/10.1038/nature03076

18. Rodriguez, A., Vigorito, E., Clare, S., Warren, M. V., Couttet, P., Soond, D. R., van Dongen, S., Grocock, R. J., Das, P. P., Miska, E. A., Vetrie, D., Okkenhaug, K., Enright, A. J., Dougan, G., Turner, M., & Bradley, A. (2007). Requirement of bic/microRNA-155 for normal immune function. Science (New York, N.Y.), 316(5824), 608–611. https://doi.org/10.1126/science.1139253

19. Thai, T. H., Calado, D. P., Casola, S., Ansel, K. M., Xiao, C., Xue, Y., Murphy, A., Frendewey, D., Valenzuela, D., Kutok, J. L., Schmidt-Supprian, M., Rajewsky, N., Yancopoulos, G., Rao, A., & Rajewsky, K. (2007). Regulation of the germinal center response by microRNA-155. Science (New York, N.Y.), 316(5824), 604–608. https://doi.org/10.1126/science.1141229

20. Thirlwell, C., Schulz, L., Dibra, H., & Beck, S. (2011). Suffocating cancer: hypoxia-associated epimutations as targets for cancer therapy. Clinical epigenetics, 3(1), 9. https://doi.org/10.1186/1868-7083-3-9

21. Vaccaro, T. D. S., Sorrentino, J. M., Salvador, S., Veit, T., Souza, D. O., & De Almeida, R. F. (2018). Alterations in the microRNA of the blood of autism spectrum disorder patients: effects on epigenetic regulation and potential biomarkers. *behavioral sciences*, *8*(8), 75.

22. Ye, Y., Hu, Q., Chen, H., Liang, K., Yuan, Y., Xiang, Y., Ruan, H., Zhang, Z., Song, A., Zhang, H., Liu, L., Diao, L., Lou, Y., Zhou, B., Wang, L., Zhou, S., Gao, J., Jonasch, E., Lin, S. H., Xia, Y., … Han, L. (2019). Characterization of Hypoxia-associated Molecular Features to Aid Hypoxia-Targeted Therapy. Nature metabolism, 1(4), 431–444. https://doi.org/10.1038/s42255-019-0045-8

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