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Role of microRNAs in Stomach Adenocarcinoma

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Abstract: ***Stomach Adenocarcinomacommonly abbreviated as STAD has emerged as the second leading cause of cancer deaths around the globe. Statistics suggest that stomach cancer, which is the fifth most common type of cancer, is presented as Stomach Adenocarcinoma in 90% of the total stomach cancer cases. The incident rate of the cancer varies according to different geographic locations, the highest incident rate is observed in Asian countries like Japan, China etc. While there has been a steady improvement in the incident rates of STAD over the past few years, the mechanism of the exact pathway has not yet been clearly understood. The development of targeted clinical therapy necessitates the precise understanding of the pathway. MicroRNAs are small non-coding RNAs, generally about 22 nucleotides long. These non-coding RNAs recognize and bind to their target RNA which then results in inhibition of translation of mRNA or its degradation.The emerging role of microRNA in various diseases such as Huntington's disease, Bipolar Disease and Parkinson's Disease etc. has attracted the attention of the scientists. Their active role in apoptosis, cell differentiation, cell growth and even carcinogenesis has clipped the attention of the researchers and efforts are constantly being put in to establish a relation (if any) between various kinds of cancers and the regulation of microRNAs. Experimental data has proved the up regulation or down regulation of microRNAs in various cancers of breast, prostate, colon, pancreas and even stomach. Through this paper we will review the role of various microRNAs in Stomach Adenocarcinoma.***

Keywords: Stomach Adenocarcinoma, microRNA, stomach cancer, molecular pathology, biomarkers

1. Introduction

Stomach Adenocarcinomaor simply STAD has emerged as the second leading cause of cancer deaths around the globe. Statistics suggest that stomach cancer, which is the fifth most common type of cancer, is presented as Stomach Adenocarcinoma in 90% of the total stomach cancer cases. The incident rates of the cancer varies according to different geographic locations, the highest incident rate is observed in Asian countries like Japan, China etc. While there has been a steady improvement in the incident rates of STAD over the past few years, the mechanism of the exact pathway has not yet been clearly understood *(Milne et al. 2007, 2009).* STAD presents itself in two sites:

- Proximal, gastro esophageal junction: 1 cm proximal and 2 cm distal region of the esophagogastricjunction.
- Fundus, body, distal and lesser or greater curvature.

These two sites are also referred as Gastric Cardia Adenocarcinoma and gastric non-cardia cancer respectively. Gastric Cardia Adenocarcinoma commonly abbreviated as GCA has rapidly increased it occurrence over the last few decades to be recognized as one of the major prevalent fatal malignancies. Due to its location of growth in the transformation zone between the esophagus and the stomach, it was formerly counted as gastric cancer or esophageal cancer. However, its distinctepidemiological and biological characteristics,it has now been diagnosed independently in recent past. GCA shares a considerable amount of similarities with esophageal squamous cell carcinoma (ESCC). The lack of specific symptoms makes the early diagnosis of the cancer almost impossible and the almost null chances of surgery in advanced conditions gives less than 25% of the patients a maximum 5 year survival frame. Current diagnostic methods include:

- Endoscopic mucosal biopsy
- Existing tumor markers, such asCEA, CA724 and CA199

While Endoscopic mucosal biopsy is an effective method of diagnosis, it is expensive and the invasiveness makes it less effective tool. The existing tumor markers on the other hand do not have enough sensitivity to be considered useful for the diagnosis of the malignancy. Hence, the histological indicators offer week prognostic value, lack of molecular markers for cellular therapy and limited effect of surgery in advanced patients, serve as three main reasons that necessitates the need of better understanding of carcinogenesis and identification of novel molecular markers.

Gene expressions are also regulated by a certain short RNA sequences that are referred to as microRNAs. Recent researches have shown that microRNAs play an important role in many diseases. Though the pattern of regulation of microRNAs has not been completely understood yet, there is enough evidence to show that they certainly affect the expression. It is well understood that microRNA recognizes semi-complementary target sequences located in 3' UTR of the mRNA. It does so by regulating a large amount of post-transcriptional repressor genes. Various cloning studies and bioinformatics experiments have estimated that microRNAs may regulate 30 % of all human genes and each microRNA can control hundreds of gene targets. About more than 2,042 microRNA sequences have already been annotated and researchers expect the numbers to double as and when more experimentation validation is carried out. The fact that, these sequences are conserved in distinctly related organisms clearly indicates that there lies important participation of microRNAs in important biological pathways that is waiting to be discovered.

The research of role of microRNAs in oncology has suggested that they can serve as a great prognostic tools mainly because their role in regulating the translation of oncogenes and tumor suppressors. Experimental data has further confirmed the presence of microRNA genes in regions of chromosome called fragile sites that control deletion or amplification.

Studies have been done on 120 microRNA associated with gastric cancer or gastric cancer cell lines. Hence, enough evidence is present to focus on microRNA research to find potential biomarkers for gastric cancer that will not only help in early detection of the disease but also will help greatly in the development of targeting gene or cellular therapies for the concerned cancer. Moreover, the identification will also pave a way for deeper understanding of carcinogenesis, thus benefitting the research and development of role of microRNAs in other kinds of cancers as well.

In subsequent sections of this review article we focus on the microRNA Biology and Function (Section 1), recent work on microRNAs in cancer (Section 2), microRNA alteration in stomach adenocarcinoma (Section 3), Mechanism of deregulation of microRNA in STAD in Section 4 followed by the Role of microRNA as biomarkers (Section 5). The article then tapers off with our conclusion (Section 6) of the research and references (Section 7).

2. Biology of microRNA and their functions:

Many researches are being carried out to elucidate the microRNAs' genomics, biogenesis, mechanism and functions etc.Aberrant microRNA expression are implicated in disease states. Thus, it has become essential to understand the biology and the functioning of microRNAs for development of targeted therapies and to devise better prognostic biomarkers of diseases.

MicroRNA genes are found in introns or exons region of other genes. They are regulated together with the host genes as they are usually found in sense orientation. Research suggest that these microRNAs are diverse in their mode of action because of the probable occurrence of RNA editing and site specific modification.

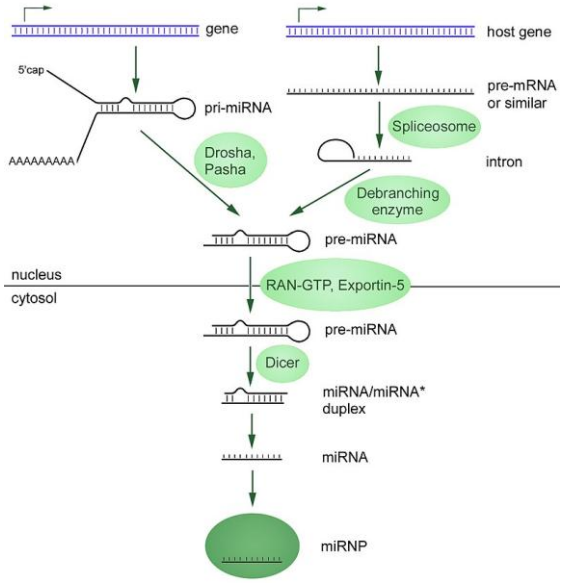

Figure: microRNA processing

The transcription of microRNA is carried by RNA polymerase 2. The process is initiated when Pol 2 binds with a promoter near the DNA sequence. Post encoding a 70 nucleotide long hair-pin loop pri-miRNA or primary microRNA is formed. The transcript is then capped at 5' end with a specialized molecule and tailed with poly Adenosines at the 3' end forming a poly-(A) tail followed by the splicing. This step is achieved by a ribonuclease named Drosha and the double-stranded-RNA-binding protein known as Pasha or DGCR8. This multi-protein complex termed as microprocessor carries on its function in the nucleus and converts the pri-miRNA to pre-miRNA or precursor-microRNA. The transcript is then transported by transporter exportin 5which is RAN GTP-dependent. From nucleus it is transported to cytosol where the transcript gets converted to the mature microRNA. The resulting 18–25-nucleotide mature microRNA then gets integrated into the RNA-induced silencing complex (RISC). The complex then exerts their regulatory effects by binding to complementary sites within the 3-untranslated region (3-UTR) of their mRNA *(Fuyi Tong, et al. 2013).*By base pairing with complementary sequences within the mRNA, microRNA works by silencing these mRNA through three typical ways:

- Cleaving the mRNA
- Destabilizing the mRNA by shortening the poly-(A) tail
- Making translation of mRNA less efficient

Other than the complementary binding with the mRNA, microRNA are found to be working as a ligand to activate signaling pathway. Research by Fabbri *et al* depicts how tumor releases certain microRNAs that bind to different receptors to induce a prometastatic inflammatory response that in turn induce tumor growth and metastasis.

In other such experiments, it is found that microRNA also function through a nuclear factor signaling pathway through interaction of natural killer cells with Tool like receptors. *(He S et al.)*

3. Recent work on microRNAs in cancer

The oncological research gives away enough evidences of presence of genes for microRNA in the region of the chromosome that regulates amplification, deletion, and fragile sites etc., all the processes involved in cancer. The deregulation of microRNA in human cancer is supported by many compelling evidences in which mechanisms such as amplification of microRNA, deletion of microRNA, abnormal transcriptional control and epigenetic changes have been observed. MicroRNAs are known to function as ***tumor suppressors or oncogene suppressors,*** and various cases have been observed where deregulation of these endogenous molecules have affected the hallmarks of cancer through one or many of the following ways:

- Sustaining proliferative signalling
- Evading growth suppressors
- Resisting cell death
- Activating invasion and metastasis
- Inducing angiogenesis

The deletion of region containing genes formiR-15 and miR-16-1 in chronic lymphocytic leukemia cells at region 13q14 of the chromosome was first identified by Dr. Croce's study that attempted to identify the tumor suppressors. This evidential study serves as the first ever concrete proof of role of microRNA. This experiment postulated that miR-15 and miR-16-1 act as tumor suppressors and deletion of the region containing these genes induced apoptosis by repressing Bcl-2. Bcl-2 is an anti-apoptotic protein which is found to be overexpressed in malignant non dividing B cells. The deletion of the region was observed in almost all cases of chronic lymphocytic leukemia. Moreover, such an experiment in rice where the region was deleted unmasked several chronic leukemia like human phenotypical symptoms in mice.

3.1 Mechanism of microRNA deregulation in Cancer

- **Amplification, deletion or translocation of microRNA genes:**

The experimental study of Dr. Croce in chronic lymphocytic leukemia suggests that the altered expression of microRNA in malignancies is often credited to the gene location and gene copy numbers of the microRNA. Through amplification, deletion or translocation of the microRNA genes owes to the tumor growth. Similar findings have been done in cases of lung cases in which deletion of the miR-143 and miR-145 results in tumor growth *(Calin GA, et al.)* The high-resolution array-based comparative genomic hybridization of human ovarian cancer, breast cancer and melanomas has provided the confirmatory analysis of the genomic alterations at the specific loci of microRNAs.

- **Alteration of transcription factors:**

Various transcription factors such as p53 control the expression of the microRNA. Hence, the alteration in the microRNAs could be because of the underlying alteration or deregulation in transcription factors.

- **Epigenetic changes:**

Genomic DNA hypomethylation, aberrant DNA hypermethylation of tumor suppressor genes and disruption of the histone modification patterns are kinds of epigenetic changes often observed in malignancies. It is stipulated that the microRNA are similarly susceptible to these epigenetic changes. The role of epigenetic modulations in microRNA expression is depicted in a lot of researches *(Donzelli S, et al.)*thus highlighting the usefulness of microRNA as biomarkers in prognosis as well as diagnosis.

- **MicroRNA biogenesis:**

As explained in section 1, the biogenesis of microRNA from gene to pri-miRNA to pre-miRNA is controlled and regulated by many enzymes including but not limited to, Drosha, Dicer, exportin 5 etc. The deregulation of any of these enzymes could result in deregulation of the biogenesis of microRNA which in turn could aid in tumor growth in malignancies. One such experimental research shows the deregulation of Drosha and dicer in cancer, thus giving concrete ground to the hypothesis. *Thomson et al.* and *Walz et al.*

4. Alteration of microRNA in stomach adenocarcinoma:

1) Researches have shown an up regulation in microRNA-223, the data has thus led scientists to believe in the role microRNA-223 as an oncogene or as a tumor suppressor gene *(Volinia S et al. 2006).*
2) Another microRNA miR-139 was found to be down regulated and its expression was found to be significantly low in the stomach cancer tissues as compared to normal tissue.Conversely, overexpression of the similar microRNA miR-139 as found to inhibit the proliferation of the cancer cell lines.*(Bao W, et al.)*
3) Another known oncogenic microRNA MiR-21, has been found to be overexpressed in the cancerous tissue. This overexpression has resulted into tumor growth through enhanced cell proliferation and invasiveness. *(Zhang Z, et al.)*

4.1 Mechanism of deregulation of microRNA in STAD:

Although a lot of studies show the deregulated microRNA in gastric cancers, yet not a clear understanding of the mechanisms of this deregulation have been obtained. As explained section 3.1 four underlying ways of deregulation have been till date studied. These are:

- Amplification, deletion or translocation of microRNA genes-
- Alteration of transcription factors
- Epigenetic changes
- MicroRNA biogenesis

The similarity of transcriptional mechanism between microRNA and protein-coding genes has led us to the conclusion that the alteration in transcription factors regulate the expression of the microRNA genes just like they regulate the expression of other genes. In a research by *Petrocca et al.* it was observed that one such microRNA miR-106b/25

cluster can be up regulated by a transcriptional factor called E2F1.

Hypermethylation and histone modification of DNA is known to have an effect on microRNA deregulation. On treatment with DNA methylation inhibitor,5-aza-20-deoxycytidine (5-Aza-CdR) and histone deacetylaseinhibitor, 4-phenylbutyric acid (PBA) the up regulation of several microRNAs located near Alu repeats on chromosome 19 *(Saito et al).* The up regulation of miR-512-5p was found to induce apoptosis in gastric cancer cells. These experiments clearly suggest epigenetic regulation of microRNAs. Several such compelling experiments support all these four underlying mechanisms of deregulation of microRNAs.

5. Role of microRNA as biomarkers in STAD

There are enough evidence to support the role of microRNA is STAD. The identification of specific microRNA can help us in standardizing few microRNAs that can act as biomarkers. This will help in numerous ways: early detection of cancer, progression monitoring and even recurrence monitoring to name a few. Their involvement in carcinogenesis and tumorigenesis is one of the main advantages that microRNA as biomarkers offer over conventional biomarkers. Furthermore, they are tissue or tumor specif. Some microRNAs as mentioned in the article above, have also shown their potential in treatment therapy and hence can be proved useful for development of targeted cell therapy for not only STAD but other cancers as well.

MicroRNAs can be easily detected and quantified in blood streams making serum-based detection of cancer possible. The ease and inexpensiveness of serum bases tests is another advantage that microRNA offers as biomarkers. *(Juan Wang, et al.)*
Another astonishing study proposes the use of microRNAs for signal transduction between cancer cells and normal cells to facilitate invasion and metastasis. This is done by using packaging circulating microRNAs in microvesicles and using them as extracellular messengers for cell-cell interaction. *(Valadi H., et al.)*

The association of microRNA deregulation with malignancies explained above in the article builds up a concrete base on the usefulness of microRNA as therapeutic agents and for development of targeted cell therapy. The two basic strategies that are being researched upon to achieve this are:

- Antagonizing the expression of microRNA using antisense technology
- Restoring the function of microRNA to exempt the expression of certain protein coding genes.

Knocking down the tumor expression of the over expressed microRNAs or induction of expression of microRNAs that have tumor suppressing affects are the two ways of tapering the growth of STAD.

6. Conclusion

The benefits and the scope of research on microRNA as prognostic, diagnostic or therapeutic biomarkers are very vast. The only issue with the present research is the inconsistency of different profiling studies. Different selection criteria, different specificity, sensitivity and selectivity of different profiling methods and different collection methods are probable reasons of the inconsistent results.

Despite, the encouraging results in the research till date there are many more challenges that lie ahead. The effect of external factors like cytotoxic treatments, pathology, hypoxia infection etc. make the research inconsistent.

However, their importance in the oncology research can't be ignore and hence more intensive research is required in this arena that is bound to give astonishing results in the field of oncology.

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