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MicroRNAs in hereditary diffuse gastric cancer (Review)

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Abstract. In 2012, gastric cancer (GC) was the third cause of mortality due to cancer in men and women. In Central and South America, high mortality rates have been reported. A total of 95% of tumors developed in the stomach are of epithelial origin; thus, these are denominated adenocarcinomas of the stomach. Diverse classification systems have been established, among which two types of GC based on histological type and growth pattern have been described as follows: Intestinal (IGC) and diffuse (DGC). Approximately 1-3% of GC cases are associated with heredity. Hereditary-DGC (HDGC), with 80% penetrance, is an autosomal-type, dominant syndrome in which 40% of cases are carriers of diverse mutations of the *CDH1* gene, which encodes for the cadherin protein. By contrast, microRNA are non-encoded, single-chain RNA molecules. These molecules regulate the majority of cellular functions at the post-transcriptional level. However, analysis of these interactions by means of Systems Biology has allowed the understanding of complex and heterogeneous diseases, such as cancer. These molecules are ubiquitous; however, their expression can be specific in different tissues either temporarily or permanently, depending on the stage of the cell. Due to the participation of microRNA in the processes of cellular proliferation, cell cycle control, apoptosis, differentiation and metabolism, these have been indicated to have a role in the development of cancerous processes, finding specific patterns of expression in different neoplasms, including GC, in which the microRNA expression profile is different in samples of non-cancerous versus cancerous tissues. A difference has been observed in the expression patterns of DGC and IGC. However, the role of microRNA in HDGC has not yet been established. The present study reviews the investigations that describe the participation of microRNA in the regulation of genes *CDH1*, *RHOA*, *CTNNA1*, *INSR* and *TGF-β* in different neoplasms, such as HDGC.

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Contents

1. Introduction

Cancer is the main cause of mortality in the world (8.2 million mortalities in 2012). Gastric cancer (GC) is estimated to be the fifth most common cancer worldwide, with 952,000 new cases annually. In 2012, GC was the third cause of mortality due to cancer in men and women; 70% of cases were reported in developing countries, with one-half of these in East Asia. In Central and South America, high mortality rates have been reported (1). In Mexico, according to the statistics of the National Institute of Statistics and Geography, the principal causes of mortalities due to neoplasms are those of the digestive tract organs (32.52 for every 100,000 inhabitants 20 years of age) (2).

GC comprises any malignant neoplasm that originates from the region between the gastroesophageal junction and the pylorus. A total of 95% of tumors developed in the stomach are of epithelial origin; thus, these are denominated adenocarcinomas of the stomach. The multifocal and polyclonal origin of the tumors renders a morphologically based histological classification complex. However, diverse classification systems have been established (3), among which the study by Lauren (4) described two types of GC based on histological type and growth pattern: Intestinal and diffuse. Intestinal- (or differentiated)-type GC (IGC) is characterized by expansive and localized growth; generally, this type of tumor type localizes in regions where an intestinal metaplasia has developed previously that, in a number of cases,

is preceded by a precancerous cascade and a prior infection with *Helicobacter pylori* (5). Diffuse GC (DGC) possesses an infiltrating pattern, is an undifferentiated adenocarcinoma and presents disperse cells with an individual, or group, invasive capacity (3).

2. Gastric cancer and heredity

Approximately 1-3% of GC cases are associated with heredity. Different syndromes have been determined of hereditary-type GC as follows: i) Lynch syndrome; ii) familial adenomatous polyposis; iii) Li-Fraumeni syndrome; iv) Peutz-Jeghers syndrome; v) juvenile polyposis syndrome; and vi) hereditary-DGC (HDGC) (6). The latter is one of the better characterized types, with 80% penetrance (7). HDGC is an autosomal-type, dominant syndrome in which 40% of cases are carriers of diverse mutations of the *CDH1* gene, which encodes for the cadherin protein (8). The International Gastric Cancer Linkage Consortium (IGCLC), based on diverse studies, has established a direct association between the mutations of the *CDH1* gene and DGC heredity; the IGCLC has additionally established various guidelines for its diagnosis, which comprise the following: i) Familial history, which is the presence of >2 cases of GC in first- or second-degree consanguinity (one confirmed case in a person aged >50 years of age), >3 cases of GC in first- or second degree consanguinity, the latter is age-independent; diagnosis of cancer <40 years of age, and a personal or familial history of DGC and/or of lobular breast cancer with an age of <50 years; ii) histopathological study; iii) detection of the mutation by genetic analysis; and iv) pathogenicity analysis (*in vitro* and *in silico*) (7). However, van der Post *et al* (9) postulated a risk population of individuals with familiar antecedents of two or more cases of bilateral lobular breast cancer, one of these in a relative with confirmed DGC presenting cleft palate and the finding *in situ* of signet ring cells, whether or not these are propagating.

3. Diffuse gastric cancer

Today, DGC comprises one of the most aggressive cancers, without defined molecular markers that permit its accurate and timely diagnosis and/or prognosis. However, *CDH1* gene mutations are associated with the development of HDGC, one-half of cases are negative for these. Thus, investigations are continuing to identify genes that are associated with HDGC. The presence of mutations has been observed in diverse genes, listed as follows: Mutations reported in *RHOA* (10,11) are associated with the initial stages of cancer and its progression to metastasis; *CTNNA1* mutated in HDGC acts as a tumor-suppressor gene (12) and changes in *MAP3K6* generate an alteration in inflammation pathways and apoptosis. In addition, it has been found that these are predisposed to develop GC (13) and that changes in the *INSR* gene (14) exert an effect on the insulin signaling pathway, giving rise to changes in the pattern of glycosylation of the E-cadherin protein, destabilization of cellular membranes and the appearance of a mesenchymal phenotype. Contrary to previously described, the roles of the mutations in the genes *FBXO24*, *DOT1L* (14) and *TGF-β* (15) have not yet been studied (Table I). Some of the mutations identified pertain to genes encoding regulatory elements or proteins that form complexes with protein E-cadherin; as a consequence of these, the loss of cellular adhesion can present due to deregulation of the complex. However, the majority of the genes postulated require studies of genetic penetrance and pathogeny in order for the genes to be established as markers.

Table I. Mutations identified in patients who were negative for mutations in the *CDH1* gene.

Gene	Protein encoded	Mutation	Refs.
RHOA	GTPase	c.50G>A c.14G>A	(10,11)
CTNNA1	A-cadherin	c.76delGA c.598G>T c.620T>G	(12)
MAP3K6	Serine/threonine protein kinase	c.2837C>T c.2872C>A c.2544delC	(13)
DOT1L	Histone methyltransferase	c.3437C>T	(14)
FBXO24	Protein 24 with F box	c.242G>C	(14)
INSR	Insulin receptor	c.3937G>A	(14)
TGF-β	TGF-β	c.29C>G	(15)

GTP, guanosine triphosphate; TGF-β, transforming growth factor-β.

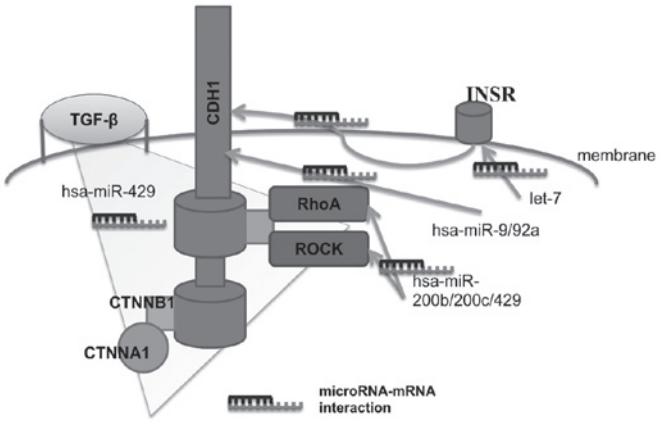

Figure 1. Role of microRNA in the regulation of mutated genes in patients with hereditary diffuse gastric cancer and involvement in the EMT. hsa-miR-9/92a microRNA binds with the 3'-untranslated region of the mRNA of *CDH1*, impeding its translation. In turn, the expression of the cadherin protein CDH1, in the EMT process can be induced by TGF-β and regulated by miR-429. The functioning of the CDH1 protein is associated with elements such as CTNNA1, RhoA and ROCK, which are regulated by the miR-200b subfamily. The gene that regulates the mRNA of INSR is controlled by the let-7 family; the protein encoded by the latter participates in the regulation of the process of E-cadherin glycosylation. EMT, epithelial-mesenchymal transition; TGF-β, transforming growth factor-β; ROCK, RhoA kinase; INSR, insulin receptor.

4. MicroRNA in HDGC

MicroRNA are non-encoded, single-chain RNA molecules (17-25 nucleotides). These molecules regulate the majority of

cellular functions at the post-transcriptional level. The regulation carried out by these molecules is complex in that they have >1 target mRNA. However, analysis of these interactions by means of Systems Biology, has allowed the understanding of complex and heterogeneous diseases, such as cancer (16).

MicroRNA are transcribed from genes and introns, individually or in groups. These molecules are ubiquitous; however, their expression can be specific in different tissues either temporarily or permanently, depending on the stage of the cell (17). Due to the participation of microRNA in the processes of cellular proliferation, cell cycle control, apoptosis, differentiation and metabolism, these have been indicated in the development of cancerous processes, by observing specific patterns of expression in different neoplasms (18-20), including GC (21-23), in which the microRNA expression profile is different in samples of non-cancerous versus cancerous tissues. A difference has been identified in the expression patterns of DGC and IGC (23). However, the role of microRNA in HDGC has not yet been established. There are studies that describe the participation of microRNA in the regulation of genes *CDH1*, *RHOA*, *CTNNA1*, *INSR*, and *TGF-β* in different neoplasms (8,10-15).

5. HDGC and genes

Beginning with the gene *CDH1*, in certain studies of microRNA functionality, it has been reported that hsa-miR-9 and hsa-miR-92a are associated with the development of metastasis in esophageal squamous cell carcinoma, their binding with the 3'-untranslated region of the *CDH1* gene, impeding the translation of the latter and induction in the epithelial-mesenchymal transition (EMT) (24,25). The cadherin complex is formed of signaling-charged E-cadherin, catenins and proteins, and the whole complex participates in the EMT. One of the genes comprising the cadherin complex is *CTNNA1*, which encodes for catenin-α. Sun *et al* (26) observed that there is a difference in the expression profile of the *CDH1*, *CTNNA1*, *CTNNB1*, *CD44* and *MMP2* genes when EMT is induced, mediated by *TGF-β*, which is regulated by hsa-miR-429, a member of the miR-200 family that has been previously associated with the EMT, on regulating zinc finger E-box binding homeobox 1/2 transcriptional repressors. Cadherin-complex signaling-mediated GTPases have regulatory elements, including RhoA and ROCK. In hepatocellular carcinoma, it was demonstrated that these two proteins are targets of the hsa-miR-200b/200c/429 subfamily; all underexpressed in the samples of analyzed tumors. By means of *in vivo* assays, it was demonstrated that deregulation of the expression profile of this miR-200b subfamily is involved in the EMT and in the development of the metastasis of this carcinoma (27). The glycosylation of E-cadherin mediated by insulin receptors (INSR) is associated with the increase in the capacity of tumor cell invasion, as well as the induction of a mesenchymal phenotype in cancerous cells (28), and it has been observed that the *INSR* gene is regulated by one of the microRNA suppressor families of tumors involved in the development of numerous neoplasms, such as let-7 (29). The regulation of the *MAP3K6*, *FBXO24* and *DOT1L* genes mediated by microRNA remains to be elucidated; however, cooperation of the protein DOT1L with c-Myc and an acetyltransferase has been described for activation of the EMT in the initiation and progression of breast cancer to metastasis (30).

6. Conclusion

In conclusion, the majority of mutations identified in patients with HDGC, such as hsa-miR-9/92 microRNA, the miR-200 family and the let-7 family, which regulate these genes, are associated with the induction of the EMT (Fig. 1). Thus, investigating the events that trigger this process in patients with HDGC is of significant importance for the establishment of genes and/or of microRNA that can be employed for the diagnosis and prognosis of this neoplasm in patients who are negative for mutations in the *CDH1* gene.

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