Original Article

J Babol Univ Med Sci
Vol 17, Issu 10; Oct 2015. P:39-45

An Investigation of the Effect of Hypoxia on Expression of 107-miR in Gastric Cancer Cell Lines MKN-45 and AGS

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Received: Feb 27th 2015, Revised: May 6th 2015, Accepted: July 29th 2015

ABSTRACT

BACKGROUND AND OBJECTIVE: Hypoxia in solid tumors is the major cause of cancer treatment resistance. Thus, identifying the appropriate indicators of hypoxia in tumors is of great importance for appropriate tumor prognosis and choosing the best treatment methods. This study aims to investigate the modulation of miR-107 expression, as a biomarker of hypoxia and its association with gastric cancer cells.

METHODS: MKN45 and AGS gastric cancer cells were purchased from Tehran Pasteur Institute and then, were cultured under normal oxygen conditions (CO_2 5% and O_2 95%) and hypoxia (CO_2 5% and N_2 95%). Finally, miR-107 and HIF-1-α expressions were evaluated every 24 to 48 hours.

FINDINGS: The results of the study showed that through hypoxia induction, HIF-1-α expression in MKN-45 cell lines increased by extending treatment duration (24 and 48 hours) 2.3 and 3.8 times, respectively, (p=0.04 and p=0.002), and in AGS cell lines HIF-1-α expression increased 2.2 and 3.8 times (p=0.03 and p=0.005). In addition, miR-107 expression in MKN-45 increased during this time by 2.2 and 3.1 times (p=0.01 and p=0.0001), while in AGS cell lines it was by 2.4 and 4 times (p=0.002 and p=0.0001).

CONCLUSION: It was demonstrated that hypoxia induction in gastric cancer cells could modulate the expression of miR-107.

KEY WORDS: Gastric cancer, HIF-1-alpha, Hypoxia, miR-107.

Please cite this article as follows:

Ayremlou N, Mozdarani H, Mowla SJ, Delavari A. An Investigation of the Effect of Hypoxia on Expression of 107-miR in Gastric Cancer Cell Lines MKN-45 and AGS. J Babol Univ Med Sci. 2015;17(10):39-45.

Introduction

Cancer is the first and second leading cause of death in the developed and developing countries, respectively. Gastric cancer is known as the fourth most common cancer worldwide and the second leading cause of death by cancer (1). This cancer is classified as a multi-factorial disease, since it is caused by infectious agents, as well as environmental and genetic factors (2). A census conducted in 2005 indicated that most cases of gastric cancer have been reported in Japan, China and Russia, while the least rate of this cancer belongs to the western developed countries (3). The

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[DOR: 20.1001.1.15614107.1394.17.10.6.1]
[DOI: 10.22088/jbums.17.10.39]

prevalence of gastric cancer differs from its mortality rate, since it is often diagnosed at advanced phases (4). Hypoxia is an important environmental feature in solid tumors (5). In this case, due to reduced blood supply, the growing tumors cells will suffer from oxygen deprivation. Unlike the common beliefs, hypoxic cells maintain their ability to grow and reproduce, they also get resistant to treatments including chemotherapy and radiation therapy. These cells will develop aggressive and metastatic behaviors (6). Hypoxia-inducible factor (HIF) is an important transcription factor which responds to changes in cellular oxygen and is activated in hypoxic conditions. When HIF is connected to hypoxia response element areas (HRE), it activates expression of around 100 genes (7). HIF target genes are the ones involved in tumor biology, that is, their proteins help with oxygen transmission, iron metabolism, glycolysis, glucose transportation, angiogenesis, cell proliferation, invasion and metastasis. (8).

HIF is consisted of HIF-1α and HIF-1-β subunits. HIF-1α regulation is dependent on oxygen, and under normal oxygen conditions, HIF-1-α is unstable, but under hypoxic conditions proline hydroxylation is inhibited, which leads to HIF-1-α stability. The stable HIF-1-α forms HIF1α–HIF1β (HIF1α–ANRT) dimer, then it is transported from the cytoplasm to the nucleus, where it binds to HRE (9). These miRNAs, which are known as the specific hypoxia-regulated miRNAs (HRM), play an important role in cell viability under low oxygen conditions (10). In studies of HRM promoter regions, the HIF binding sites were identified.

It was also found that approximately 6% of the human miRNAs have HIF binding sites which are among the 17 protected species, indicating the importance of these sites. Since the role of miRNA as an effective factor in development of gastric cancer has been identified, miRNA expression profiles have been extensively analyzed using microarray, real-time PCR and Next Generation Sequencing. A number of miRNA functional mechanisms have been identified in the process of development of gastric cancer.miRNA can be influential not only in tumors induced by oncogenes but also in tumors created by suppressing tumor genes . Currently, the role of miRNA in diagnosis and treatment of gastric cancer has been widely studied. miR-107 is placed on 10q23.31 chromosome and has a variety of pathways including adipogenic cells, cell cycle, hypoxia, angiogenesis and neurodegenerative diseases, and there are extensive evidence regarding oncogenic role of miRNA.

Overexpression of miR107 has been reported in some malignancies, including gastric, breast, pancreatic and colorectal (14-11). Moreover, miR-107 expression changes level have been reported in various cancers, which is significantly associated with the development and progression of cancer. However, its role in gastric cancer has not been fully understood yet. Low-expression of miR107 has been reported in some cancers. Many other studies also reported the overexpression of miR-107 in gastric (15, 13), esophagus (16) and liver (17) cancers demonstrating its positive role in carcinogenesis. Overexpression of miR-107 has been reported as a gastric cancer oncogene and regulator of tumor invasion and metastasis. Recently, Li and colleagues have shown overexpression of miR107 in the gastric cancer and suggested that this raise in expression is associated with the clinical progression of the cancer and increased cell proliferation by targeting 1FOX (18). The differences in the rise high and fall of low expression level and the role of miR-107 in various cancers is yet to be discovered, but it is probable that miR-107 role is specific to cell or tissue proliferation, downstream targeted genes and contents of different cells in different tissues.

As mentioned above, the presence of hypoxia in solid tumors will increase the expression of some genes, and ultimately, it will lead to tumor aggressive behavior, such as metastasis, poor

[DOR: 20.1001.1.15614107.1394.17.10.6.1]
[DOI: 10.22088/jbums.17.10.39]

prognosis and lack of response to current treatments, including surgery, chemotherapy and radiation therapy. Patients suffering from this disease require a series of special treatment such as the use of some chemical substances which decreases the aggressive behavior. Thus, the initial identification of hypoxia in tumor may be useful in choosing the most effective treatment. However, the study of markers in biopsy tissue of tumor is quite invasive and expensive.

Therefore, identifying the non-invasive biomarkers such as discharging miRNAs in serum and plasma, is essential for detecting of tumor hypoxia. While increased expression of miR107 has been previously reported in gastric cancer, the hypoxia's impact on the increased expression has not been studied yet. This study aims to investigate changes of miR-107 expression in MKN45 and AGS gastric cancer cell lines through induction of hypoxia.

Methods

Cell culture and hypoxia induction: MKN45 and AGS gastric cancer cell lines were provided from the Pasteur Institute of Iran. Cancer cell lines were cultured in an environment consisting of RPMI-1640, 10% FBS, 1% pen strep at 37°C and 5% CO_2 incubator. To induce hypoxia, cell lines were cultured in N_2 95% and CO_2 5%. Total cell RNA extraction: In order to investigate the expression of HIF-1α and miR-107, we first began to extract total cellular RNA from gastric cancer cell units by RiboEX solution (GeneAll, Korea), which was done according to the manufacturer's instructions. The concentration and purity of the extracted RNA were determined by means of a spectrophotometer (GeneQuest, UK).

The HIF-1α expression measurement through real-time PCR method: Reversed transcription reaction on 500 ng RNA was carried out using cDNA synthesis (Takara, Japan) kit. Micro tubes were incubated for 15 minutes at 37ºC, then in order to deactivate the enzymes, they were re-incubated for 5 minutes at 85ºC. We employed ExiLent SYBR Green master mix (Takara, Japan) kit to perform real-time PCR reaction. GAPDH was used as the internal control for normalization. PCR products were reproduced according to the instructions, that is a 30 seconds at 95ºC followed by 40 cycles of 95ºC for 3 minutes and 60 seconds in a 60ºC ABI 7500 real-time quantitative PCR system (Applied biosystems, USA). To ensure lack of the contamination safety with genomic DNA and verification of the negative control, an RT and cDNA free reaction was applied.

HIF-1α (F-TGACCTGCTTGGTGCTGATT/R-AGCGGCCTAAAAGTTCTTCTG)
GAPDH (F- ATGGGGAAGGTGAAGGTCG/R-GGGGTCATTGATGGCAACAATA)

miR-107 level measurement USING THE Real-time PCR method: reverse transcription reaction on 200 ng RNA was performed using a miRCURY LNA™ Universal RT microRNA PCR Kit (Exiqon, Denmark). Micro tubes were incubated for 60 minutes at 42ºC , then to deactivate the enzymes they were re-incubated for 5 more minutes at 95ºC. In order to perform real-time PCR reaction through SYBR Green method, 1 µg of cDNA, miR-107 LNA™ primers and miRCURY LNATM Universal RT microRNA PCR Kit (Exiqon, Denmark) were used. srRNA 5 was also employed for internal control normalization. PCR products were reproduced according to the temperature timetable, which is consisted of 10 minutes at 95ºC followed by 40 cycles (10sec) within 95ºC and finally, 1 minute inside the ABI 7500 real-time quantitative PCR system at 60ºC (Applied biosystems, USA).

Each - expression experiment was repeated twice. To ensure the contamination safety with genomic DNA and verification of the negative control, an RT and cDNA free reaction was used in each experiment.Statistical data analysis: The

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expression of HIF-1α and miR-107 in gastric cancer cell samples was analyzed using Graphpad Prism 6 software and $2^{-\Delta\Delta CT}$ method. To assess the significance of differences, T student test was used and P-value less than 0.05 was considered significant.

Results

HIF-1α expression in MKN45 and AGS gastric cancer cell lines: The statistical analysis showed that hypoxia induction in MKN45 and AGS cell lines increase the expression of HIF-1α. The amount of HIF-1α in MKN45 cells within 24 and 48 hours after hypoxia induction, as compared to normal oxygen condition, was increased 2.3 and 3.8 times, respectively. The expression demonstrated a significant difference at 24- and 48-hour intervals, respectively (p=0.04 and p=0.002) (Figure 1). In addition, the expression of HIF-1α in AGS cells 24 and 48 hours following induction of hypoxia had increased 2.2 and 3.8 times, respectively. During this time period, the expression also showed a significant difference, as compared to normal oxygen conditions (p=0.005 and p=0.03, respectively) (fig 1). MiR-107 expression in MKN45 and AGS gastric cancer cell lines: miR107 expression in MKN45 cells, which were cultured under hypoxic conditions, increased.

They also manifested an expansion 24 and 48 hours following induction of hypoxia (in comparison to normal oxygen conditions) by 2.2 and 3.1 times. The expression within 24 and 48 hours again showed a significant difference, as compared to normal oxygen condition (p=0.0001 and p=0.002, respectively) (fig 2). Replicating the experiment for creation of AGS also represented an increase in miR-107 expression after 24 and 48 hours of hypoxia induction by 2.8 and 4 times. Expression of this cell line within 24 and 48 hours, as compared to normal oxygen conditions were significantly different (p=0.0001 and p=0.002, respectively) (fig 2).

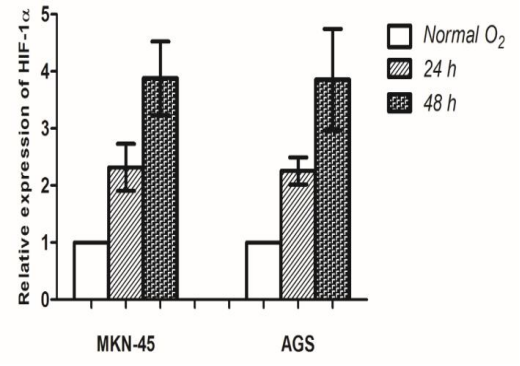


Figure 1. A comparison of HIF-1α expression in MKN45 and AGS cells in hypoxic conditions

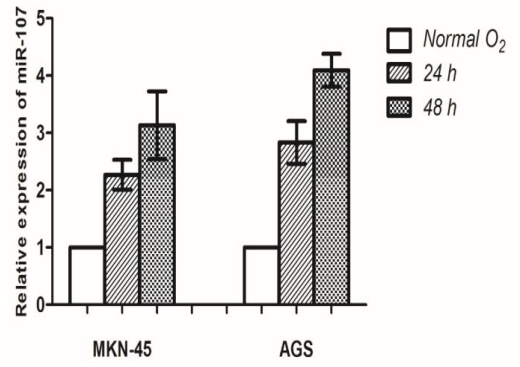


Figure 2. A comparison of miR-107 expression in MKN45 and AGS cells in hypoxic conditions

Discussion

In this study, the induction of hypoxia in the gastric cancer cell lines led to increased expression of miR-107, moreover, by increasing hypoxia time from 24 hours to 48 hours HIF-1α expression was enhanced as well. HIF-1α is one of the HIF subunits, which is unstable in normal oxygen conditions and decomposes as a result. But in hypoxic situations, which are the characteristic of solid tumors, it remains stable and causes particular behaviors in tumor cells (9).

Therefore, following induction of hypoxia we an increased expression of HIF-1α is expected, demonstrating approval of an indication of hypoxia-induced in cell lines MKN45 and AGS. Several studies have proven the various roles of miRNA as regulators of cellular functions, oncogenes or tumor suppressor in the development

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and progression of tumors (19). Several studies led to identification of a group of miRNAs, which are regulated by hypoxia (HRMs). HRM group is consisted of 93, 103, 30b, 27a, 26b, 26a, 24, 23b, 23a, miR-21, 213, 210, 195, 192, 181abc, 125b, 107, 106a, which are induced as a response to hypoxia in breast and clonal cancers (10).

Although miRNA studies are widely done in various cancer types, but there are few studies on changes and functions of miRNA in gastric cancer. miR-107 was initially identified as a repressive factor of tumors in bladder, pancreas, colon, head and neck cancers (21, 20, 14). The increase in expression in pancreatic cancer cells results in reduced levels of CDK6 and inhibition of cell growth (14). It has been also proven that miR-107 in gastric cancer, by targeting CDK6, keeps the cells in the G1 cell cycle phase and thereby inhibits tumor invasion (22). In addition to the abovementioned factors, miR-107 inhibited the development of colon cancer (21). The significant growth of miR-107 in non-ductal tumor cells leads to pancreatic cancer (23).

However, the results of numerous studies on the role of miR-107 mark it as an oncogene in breast cancer (24, 25). miR107 expression growth is associated with metastasis and poor prognosis of breast cancer. miR-107 expression growth in mammary epithelial cells, turns them into mesenchymal cells, which eventually leads to metastatic behavior through the DICER1 (24) Several studies have demonstrated overexpression of miR-107 in various types of cancer, including stomach, esophagus, colon, liver and pancreas. This kind of miRNA has been suggested to be a positive factor in cancer development (17-13).

MiR-107 has been identified as an miRNA regulating invasion and metastasis in gastric cancer (13). Inoue and colleagues have indicated the increased expression of miR-107 in gastric tumor tissue, as compared to adjoining healthy tissue. They also showed that its expression is directly associated with the rate of invasion, lymph node metastasis and tumor grade (26). Li and colleagues found increased expression of miR-107 in gastric tumor, they suggested its association with clinical progression and increased tumor cell growth through FOXO1 (18).

As mentioned above, increased and decreased expression of miR107 in variety a of tumors have been mentioned. Although the reason for this contradiction is unclear, but there is no doubt that the role of -miR107 in cell growth is dependent on the tissue and cell, which can be different as a result of intracellular factors and targeted genes. Therefore, the role and mechanism of miR-107 in a variety of tumors requires further studies and using special tissue modeling. In general, tumors with hypoxia have poor prognosis and are resistant to current treatments such as chemotherapy and radiation. Hence, for the treatment of these patients using a series of special treatments, such as genes involved in development of hypoxic tumor behaviors are used.

Through mediation of epigenetic pathways, miRNA helps with creation of new phenotypes (27). miR-107 is one of miRNAs induced in response to hypoxia within cancer cells; however, very few studies have been conducted on the relationship of hypoxia with its expression. miR-107 expression growth is monitored in response to hypoxia in breast and colon cancer cells (10). Although gastric cancer is one of the most common solid tumors and hypoxia is one of its distinctive features, but no reports have been provided regarding the effects of hypoxia on 107-miR expression in gastric tumor cells. In this study, the relationship between hypoxia and miR-107 increased expression in MKN45 and AGS gastric cancer cells were identified.

Based on these findings, we can conclude that miR107, as a biomarker of the diagnosis of gastric cancer, can help with determining tumor hypoxic situation and choosing an appropriate treatment for gastric hypoxic tumor. Yet further extensive studies are required on this issue.

[DOR: 20.1001.1.15614107.1394.17.10.6.1]
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Acknowledgments

This Study was supported by the Research Department of the Tarbiat Modarres University, Tehran, Iran.

References

1.Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893-917.

2.Zabaleta J. Multifactorial etiology of gastric cancer. Methods Mol Biol. 2012;863:411-35.

3.Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J. 2005;81(957):419-24.

4.Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000;343(2):78-85.

5.Vaupel P, Hockel M, Mayer A. Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal. 2007;9(8):1221-35.

6.Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst. 2001;93(4):266-76.

7.Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer.2003;3(10):721-32.

8.Poon E, Harris AL, Ashcroft M. Targeting the hypoxia-inducible factor (HIF) pathway in cancer. Expert Rev Mol Med. 2009;11:e26.

9.Yu F, White SB, Zhao Q, Lee FS. HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc Natl Acad Sci U S A. 2001;98(17):9630-5.

10.Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, et al. A microRNA signature of hypoxia. Mol Cell Biol. 2007;27(5):1859-67.

11.Li XY, Luo QF, Wei CK, Li DF, Li J, Fang L. MiRNA-107 inhibits proliferation and migration by targeting CDK8 in breast cancer .Int J Clin Exp Med. 2014;7(1):32-40.

12.Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, Huang J, et al. miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4. Cancer Res. 2012;72(14):3631-41.

13.Li X, Zhang Y, Shi Y, Dong G, Liang J, Han Y, et al. MicroRNA-107, an oncogene microRNA that regulates tumour invasion and metastasis by targeting DICER1 in gastric cancer. J Cell Mol Med. 2011;15(9):1887-95.

14.Lee KH, Lotterman C, Karikari C, Omura N, Feldmann G, Habbe N, et al. Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. Pancreatology. 2009;9(3):293-301.

15.Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257-2261.

16.Guo Y, Chen Z, Zhang L, Zhou F, Shi S, Feng X, et al. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. Cancer Res. 2008;68(1):26-33.

17.Huang XH, Wang Q, Chen JS, Fu XH, Chen XL, Chen LZ, et al. Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated. Hepatol Res. 2009;39(8):786-94.

18.Li F, Liu B, Gao Y, Liu Y, Xu Y, Tong W, et al. Upregulation of microRNA-107 induces proliferation in human gastric cancer cells by targeting the transcription factor FOXO1. FEBS Lett. 2014;588(4):538-44.

19.Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer. 2006;94(6):776-80.

20.Datta J, Smith A, Lang JC, Islam M, Dutt D, Teknos TN, et al. microRNA-107 functions as a candidate tumor-suppressor gene in head and neck squamous cell carcinoma by downregulation of

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[DOI: 10.22088/jbums.17.10.39]

protein kinase Cvarepsilon. Oncogene. 2012;31(36):4045-53.

21. Yamakuchi M, Lotterman CD, Bao C, Hruban RH, Karim B, Mendell JT, et al. P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. Proc Natl Acad Sci U S A. 2010;107(14):6334-9.

22. Feng L, Xie Y, Zhang H, Wu Y. miR-107 targets cyclin-dependent kinase 6 expression, induces cell cycle G1 arrest and inhibits invasion in gastric cancer cells. Med Oncol. 2012; 29(2): 856-63.

23. Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol. 2006;24(29):4677-84.

24. Chen PS, Su JL, Cha ST, Tarn WY, Wang MY, Hsu HC, et al. miR-107 promotes tumor progression by targeting the let-7 microRNA in mice and humans. J Clin Invest. 2011;121(9):3442-55.

25. Cicatiello L, Mutarelli M, Grober OM, Paris O, Ferraro L, Ravo M, et al. Estrogen receptor alpha controls a gene network in luminal-like breast cancer cells comprising multiple transcription factors and microRNAs. Am J Pathol. 2010;176(5):2113-30.

26. Inoue T, Iinuma H, Ogawa E, Inaba T, Fukushima R. Clinicopathological and prognostic significance of microRNA-107 and its relationship to DICER1 mRNA expression in gastric cancer. Oncol Rep. 2012;27(6):1759-64.

27. Sassen S, Miska EA, Caldas C. MicroRNA: implications for cancer. Virchows Arch. 2008;452(1):1-10.

[DOR: 20.1001.1.15614107.1394.17.10.6.1]

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