Review Article

RhoA mutations in diffuse-type gastric cancer

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Abstract: Gastric cancer is one of the most common malignant tumors worldwide. According to the Lauren classification, gastric cancer can be divided into three types: intestinal-type gastric cancer (IGC), diffuse-type gastric cancer (DGC), and mixed type. Compared with IGC, DGC is more malignant and has a worse prognosis. Ras homolog gene family member A (RhoA) protein is a member of the Rho family of small guanosine triphosphate (GTP)-binding proteins and is encoded by the gene *RhoA*. It acts as a molecular switch in cell signaling and takes part in a variety of cellular biological processes. *RhoA* mutations are closely related to the occurrence and development of DGC. This article reviews the recent advances in the role of *RhoA* mutations in DGC, with an attempt to renew our understanding of DGC.

Keywords: *RhoA*; mutation; diffuse-type gastric cancer (DGC)

Received: 06 January 2020; Accepted: 21 January 2020; Published: 25 February 2020.
doi: 10.21037/dmr.2020.01.03
View this article at: http://dx.doi.org/10.21037/dmr.2020.01.03

Gastric cancer is one of the most common malignant tumors worldwide. According to the GLOBOCAN estimates of incidence and mortality worldwide for cancers, there were 1.03 million new cases of gastric cancer and 780,000 deaths in 2018. Gastric cancer was the fifth most common malignancy and the third leading cause of cancer-related death, coming only after lung cancer and colorectal cancer (1). China is one of the countries with the highest incidence of gastric cancer and accounts for over nearly half of all new gastric cancer cases in the world (2). According to the Report of Cancer Incidence and Mortality in Different Areas of China released by the National Cancer Center of China in February 2018, the incidence and mortality rate of gastric cancer ranked the second and third places among all malignancies in China (3).

Diffuse-type gastric cancer (DGC)

Gastric cancer is a highly heterogeneous disease. Different degrees of differentiation and biological behaviors can often be seen among different subtypes and even in different regions of the same tumor. Many gastric cancer classifications have been proposed to improve the prognosis of gastric cancer by achieving personalized treatment and guide clinical decision-making. According to the Lauren classification, gastric cancer can be divided into three types: intestinal-type gastric cancer (IGC), DGC, and mixed type (4). Among them, DGC accounts for about 22% and 50%, which varies significantly in different areas (5). Lauren classification is based on differences in tumor histopathology and biological behaviors. Compared with IGC, DGC cells are less adhesive and highly aggressive; the cancer is poorly differentiated and becomes metastatic in its early stages; it can occur in younger populations, with high recurrence rate and poor prognosis (6,7); morphologically, DGC mostly corresponds to the type III (ulcerating growth) or type IV (diffusely infiltrating growth) in the Borrmann classification.

The Lauren classification is clinically valuable for predicting the prognosis of patients with gastric cancer. In

a retrospective study, Chen *et al.* even believed Lauren's classification was an independent prognostic factor for gastric cancer (7). However, the role of the Lauren classification in the personalized treatment of gastric cancer, screening of potential drug targets, and prediction of the efficacy of molecularly targeted cancer therapy. It is well believed that the pathogenesis of IGC is affected by environmental factors, while the leading causes of DGC are genes and heredity factors. With the development of gene chip and sequencing technology, gastric cancer research has entered the era of molecular omics, and many new potential therapeutic targets have been discovered. Thus, a new gastric cancer classification urgently must achieve more precise management of gastric cancer patients. The Cancer Genome Atlas (TCGA) has performed a comprehensive analysis in 295 chemoradiotherapy-naïve gastric cancer patients and uncovered four molecular subtypes of gastric cancer: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN). It was found that the GS tumors were diagnosed at an earlier age (median age 59 years), along with higher possibilities of *CDH1* and *RhoA* gene mutations and CLDN18-ARHGAP26 fusions (8), which morphologically corresponds to the DGC in the Lauren classification.

Structure and function of RhoA protein

RhoA, a 21-kDa guanylate-binding protein, is encoded by the *RhoA* gene and found in human chromosome 3p21.3. RhoA protein is a member of the Rho subgroup that belongs to the Ras superfamily. The protein was first discovered and cloned in 1985 (9), and its function in regulating cytoskeleton was first confirmed in 1995 (10). RhoA is one of the typical members of the Rho family.

RhoA protein has a structural domain that binds to guanosine triphosphate (GTP). With the GTPase activity, it can hydrolyze GTP into guanosine diphosphate (GDP). The RhoA protein is activated when binding to GTP and can activate the downstream signaling molecules through allosteric effect, opening the corresponding signaling pathways; in contrast, RhoA protein is in an inactive state when it binds to GDP, which can cause downstream signaling pathways to be closed (11). Guanine nucleotide exchange factors (GEFs) activate monomeric GTPases by stimulating the release of GDP to allow binding of GTP; GTPase-activating proteins (GAPs) accelerate the GTP hydrolysis mediated by GTPases and thus inactivate the RhoA protein (12). Guanine nucleotide dissociation inhibitors (GDIs) have dual functions: When GDIs exist in a dissolved state in the cytoplasm, it can prevent the transformation of RhoA from a GDP-bound state to a GTP-bound state; when GDIs are located on the cell membrane, they can interact with RhoA-GTP to promote GTP hydrolysis.

As a molecular switch in cell signaling, RhoA protein takes part in many biological functions of the cells by cycling between these two different conformational states. After the RhoA protein is activated, it can activate the downstream protein kinase ROCK1 and protein kinase N, thereby phosphorylating and inactivating the myosin phosphatase and increasing the level of phosphorylated myosin light chain in the cytoplasm, leading to the increased cross-linking between myosin and kinesin, which promotes the aggregation of cytoskeleton and the shrinkage, adhesion, proliferation, apoptosis, and migration of cells (13,14). RhoA protein can also regulate the polymerization of actin monomers into actin through ROCK1 and mDia pathways (15).

Mechanisms of RhoA mutations in DGC

The RhoA signaling pathway takes part in multiple life processes by regulating the cytoskeleton. Therefore, abnormal RhoA signaling pathways and *RhoA* mutations or abnormal expression have been reported to be closely associated with the occurrence and development of a variety of diseases, including malignant tumors (16-18). Although the expression of RhoA is increased in multiple tumors, *RhoA* mutations are less common in tumors (19,20). Kakiuchi *et al.* performed whole-exome sequencing on 87 DGC cases and found recurrent *RhoA* nonsynonymous mutations in 22 cases (25.3%), with mutational hotspots including p.Tyr42Cys (from tyrosine to cysteine at position 42), p.Gly17Glu (from glycine to glutamic acid at position 17), and p.Arg5Gln (from arginine to glutamine at position 5); subsequent comparison with the sequencing results in 51 patients with IGC showed that *RhoA* mutations specifically occurred in DGC (21). The study performed by Wang *et al.* (22) also identified *RhoA* mutations in 14.3% of DGC patients, with the two most common mutation hotspots being p.Tyr42Cys, and p.Leu57Val (from leucine to valine at position 57); further analysis showed that most *RhoA* mutations occurred in the functional domains where RhoA bound to substrate protein or GTP; in particular, four hotspots including p.Tyr34Cys (from tyrosine to cysteine at position 34), p.Phe39Val (from phenylalanine to valine

at position 39), p.Glu40Val (from glutamic acid to valine at position 40), and p.Tyr42Cys were located in the domains where RhoA bound to substrate proteins. By using a new molecular classification of gastric cancer, TCGA found that *RhoA* mutations often occurred in the GS group and that RhoA mutations were not similar to the oncogenic mutations of the Ras family GTPase genes (8).

The functional changes of *RhoA* mutants are still controversial. Kakiuchi *et al.* found that the distribution of mutation positions was uneven and speculated that *RhoA* mutations might gain new functions. Thus, the authors studied cancer cell lines harboring *RhoA* mutations: the OE19 cell line (adenocarcinoma of the gastric cardia) harboring p.Tyr42Cys mutation, the breast cancer cell line BT474 harboring p.Gly17Glu mutation, and the colorectal cancer line SW948 harboring p.Gly17Glu mutation. Gastric cancer cell lines (AGS and MKN74) containing wild-type *RhoA* were used as controls. When small interfering RNA (siRNA) was used to silence the expression of the *RhoA* gene, the growth of OE19, SW98, and BT474 cell lines was significantly suppressed, while the growth of the wild-type gastric cancer cell lines AGS and MKN74 were not affected.

Furthermore, the authors performed functional rescue experiments in the growth-suppressed SW948 cells, in which the p. Tyr42Cys RhoA mutant and p. Gly17Glu RhoA mutant and wild-type *RhoA* gene were separately introduced. It was found that the inhibitory effect on SW948 disappeared when the mutant was introduced; in contrast, the remarkable growth-inhibiting effect persisted in cells introduced with the wild-type gene. Thus, *RhoA* mutations specifically promote the growth of tumor cells, which may depend on the tumor-specific environments and signaling pathways. Accordingly, the authors believed that the gain-of-function of *RhoA* mutations plays a vital role in the biological behaviors of DGC (21).

However, Wang *et al.* (22) believed that RhoA mutations (especially those in four hotspots including p.Tyr34Cys, p.Phe39Val,p.Glu40Val, and p.Tyr42Cys) caused the functional loss by analyzing RhoA mutation patterns and combining with previous studies (23,24). Therefore, the authors performed experiments on 293T/17 cells expressing p.Tyr42Cys, and p.Leu57Val genes, respectively, and found that, compared with wild-type 293T/17 cells, the number of activated RhoA proteins in cells with mutations at positions 42 and 57 were significantly reduced. Physiologically, the RhoA signaling pathway mediates anoikis, and anti-anoikis is a crucial step in the progression of DGC. The authors used mouse intestinal organoids to further investigate the effects of *RhoA* mutations on anoikis and oncogenesis. First, they made the organoids express wild-type *RhoA* and mutant *RhoA* (p. Tyr42Cys and p. Leu57Val, respectively), and a blank control group was also used. Then, the organoids were dissociated into a single cell suspension by using tryptase. After 4 days of culture in the presence of ROCK inhibitor, it was found that the organoid cells expressing mutant *RhoA* remarkably "restored" the organoids, while the cells expressing wild-type *RhoA* failed to re-generate organoids. In the absence of ROCK inhibitor, both cells in the blank control group and wild-type cells died on the 10th day of culture, while *RhoA* mutant cells continued to grow (22). Thus, *RhoA* mutations lead to the loss of RhoA function, thus enabling the cells to gain the ability to resist anoikis, which promotes the infiltration and diffuse growth of cells.

In TCGA's study, *RhoA* mutations activated the downstream ROCK protein. The product of CLDN18 gene expression is one of the components of tight cell junctions. The outcomes of ARHGAP26 gene expression are GAPs. The fusion genes between CLDN18 and ARHGAP26 reported in TCGA were found between exon 5 of CLDN18 and either exon 10 or 12 of ARHGAP26. Such fusion genes specifically appeared in *RhoA* mutation cases; mRNA sequencing revealed a mature fusion protein product. Determination of the gene expression status in the signaling pathway suggested that *RhoA* mutation activated the RhoA signaling pathway. Since RhoA plays a central role in cell migration, the *RhoA* mutant and the CLDN18-ARHGAP26 fusion may be involved in the aggressive behaviors of DGC (8). Zhang H *et al.* cultured the normal intestinal epithelium of mice into organoids and knocked out the organoid *CDH1* gene and overexpressed the *RhoA* mutation (p. Tyr34Cys) gene *in vitro*, thus successfully inducing the normal organoids into DGC. It demonstrated that *RhoA* mutations gained new functions and therefore played critical roles in the pathogenesis of DGC (25). Nishizawa *et al.* (26) performed a study in gastric cancer cell lines with different mutation sites and demonstrated that *RhoA* mutations inactivated the ROCK protein, thereby maintaining cell survival and inducing cell migration.

Relationship of RhoA mutations with the clinical features of DGC

RhoA mutations specifically occur in DGC and are believed to promote the progression of this malignancy. Therefore,

we speculated that the *RhoA* mutations are associated with the gender and age of the DGC patients and with the location, type, stage, and prognosis of cancer itself. Ushiku *et al.* (27) retrospectively analyzed 87 cases of DGC and grouped the patients by *RhoA* mutations (or not) to explore the relationship between *RhoA* mutations and clinical features of DGC. They found that *RhoA* mutations were not significantly associated with the age of disease onset or gender. Of the 22 patients with *RhoA* mutations, 16 had advanced DGC, among which 13 (81%) were Borrmann type III, 3 (19%) were Borrmann type IV, and the remaining 6 were still in their initial stages. Wang *et al.* also identified *RhOA* mutation in a case of intramucosal cancer (22). These findings suggest that *RhoA* mutations may play a vital role in the preliminary stages of DGC. Further histological studies showed that 73% of *RhoA*-mutant cases had locally differentiated tubular components in the gastric mucosa. Kakiuchi *et al.* sequenced the mucosal tubular components and the deeper invasive poorly cohesive component and found the *RhoA* mutations were present in both components (21), which further demonstrated that *RhoA* mutations might occur in the early stages of gastric cancer.

A preliminary study did not find significant differences in the stage and prognosis of DGC between *RhoA*-mutant group and wild-type group (27). However, it was believed that the study had the following limitations that lead to the negative results: (I) the sample size was small; (II) since Borrmann type IV patients were included in the study, the heterogeneity of gastric cancer might have affected the patients' prognoses; and (III) no other abnormal changes (e.g., CLDN18-ARHGAP26 fusion) in the RhoA signaling pathway were included in the analysis. Therefore, the relationship between *RhoA* mutations and the stage and prognosis of gastric cancer still requires further studies with larger sample sizes (27).

RhoA mutations and DGC treatment

As described above, compared with IGC, DGC is more malignant and has a worse prognosis. Currently, multidisciplinary treatment (mainly surgical resection) is still the mainstream treatment for gastric cancer. Despite the advances in a variety of treatment methods, the outcomes of gastric cancer patients are far from satisfactory. For patients with human epidermal growth factor receptor 2 (HER2)-positive gastric cancer, postoperative chemotherapy combined with anti-HER2 therapy is currently the only proven targeted therapy for gastric cancer (28). According to Kakiuchi *et al.*, however, the HER2-positive type accounted for only 4.5% of *RhoA*-mutant gastric cancer (21), and its positive rate was also below 10% among all DGC cases (29). Thus, new molecularly targeted drugs for DGC are urgently needed. *RhoA* mutations specifically occur in DGC and are closely related to the occurrence and development of this malignancy; in contrast, *RhoA* is highly conserved in normal cells. Thus, it can be used as a potential therapeutic target. For example, a targeted drug may be designed to affect the binding of *RhoA* mutants to GTP or the interaction between RhoA and regulatory proteins (e.g., GAPs and GEFs) without worrying about its effect on normal cells.

Summary

In summary, *RhoA* mutations are not common in malignant tumors, but they specifically occur in DGC. The functional changes after *RhoA* mutations are still controversial, although functional defects or new functions may develop, as proved in biochemical studies. In terms of the biological behaviors of DGC, it is currently believed that *RhoA* mutations promote the occurrence and development of gastric cancer through various known and unknown mechanisms and thus play the roles of oncogenes (30). However, studies with limited sample sizes did not find the impact of *RhoA* mutations on the prognosis of DGC, and thus *RhoA* mutations are still not a prognostic factor. *RhoA* mutation may become a potential therapeutic target for GDC, which may improve the prognosis of DGC patients and achieve personalized treatment. Further research is still needed to elucidate how *RhoA* mutants play a carcinogenic role in the tumor environments, and rationally designed studies with larger sample sizes are warranted to clarify the relationship between RhoA *mutations* and clinical features of DGC.

Acknowledgments

Funding: This work was supported by the Science and Technology Planning Project of Guangdong Province, China (2017A020215014) and the Medical Scientific Research Foundation of Guangdong Province, China (A2017274).

Footnote

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at http://dx.doi.org/10.21037/dmr.2020.01.03). YH serves as the Editor-in-Chief of *Digestive Medicine Research*. CZ serves as the unpaid Associate Editor-in-Chief of *Digestive Medicine Research*. The other authors have no conflicts of interest to declare.

Ethical Statement: All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
3. Chen W, Sun K, Zheng R, et al. Report of Cancer Incidence and Mortality in Different Areas of China, 2014. China Cancer 2018;27:1-14.
4. Lauren P. The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. An Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand 1965;64:31-49.
5. Hayashi T, Yoshikawa T, Bonam K, et al. The superiority of the seventh edition of the TNM classification depends on the overall survival of the patient cohort: comparative analysis of the sixth and seventh TNM editions in patients with gastric cancer from Japan and the United Kingdom. Cancer 2013;119:1330-7.
6. Stiekema J, Cats A, Kuijpers A, et al. Surgical treatment results of intestinal and diffuse type gastric cancer. Implications for a differentiated therapeutic approach? Eur J Surg Oncol 2013;39:686-93.
7. Chen YC, Fang WL, Wang RF, et al. Clinicopathological Variation of Lauren Classification in Gastric Cancer. Pathol Oncol Res 2016;22:197-202.
8. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-9.
9. Madaule P, Axel R. A novel ras-related gene family. Cell 1985;41:31-40.
10. Ridley AJ, Comoglio PM, Hall A. Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. Mol Cell Biol 1995;15:1110-22.
11. Kawano Y, Kaneko-Kawano T, Shimamoto K. Rho family GTPase-dependent immunity in plants and animals. Front Plant Sci 2014;5:522.
12. Alberts B, Johnson A, Lewis J. Molecular Biology of the Cell. Sixth Edition. Garland Science, 2014.
13. Leung T, Manser E, Tan L, et al. A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem 1995;270:29051-4.
14. Thumkeo D, Watanabe S, Narumiya S. Physiological roles of Rho and Rho effectors in mammals. Eur J Cell Biol 2013;92:303-15.
15. Watanabe N, Madaule P, Reid T, et al. p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. Embo J 1997;16:3044-56.
16. Shimokawa H, Sunamura S, Satoh K. RhoA/Rho-Kinase in the Cardiovascular System. Circ Res 2016;118:352-66.
17. Zhou J, Hayakawa Y, Wang TC, et al. RhoA mutations identified in diffuse gastric cancer. Cancer Cell 2014;26:9-11.
18. Palomero T, Couronne L, Khiabanian H, et al. Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. Nat Genet 2014;46:166-70.
19. Karlsson R, Pedersen ED, Wang Z, et al. Rho GTPase function in tumorigenesis. Biochim Biophys Acta 2009;1796:91-8.
20. Faried A, Faried LS, Usman N, et al. Clinical and prognostic significance of RhoA and RhoC gene expression in esophageal squamous cell carcinoma. Ann Surg Oncol 2007;14:3593-601.
21. Kakiuchi M, Nishizawa T, Ueda H, et al. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. Nat Genet 2014;46:583-7.
22. Wang K, Yuen ST, Xu J, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. Nat Genet 2014;46:573-82.
23. Sahai E, Alberts AS, Treisman R. RhoA effector mutants

reveal distinct effector pathways for cytoskeletal reorganization, SRF activation and transformation. EMBO J 1998;17:1350-61.
24. Bae CD, Min DS, Fleming IN, et al. Determination of interaction sites on the small G protein RhoA for phospholipase D. J Biol Chem 1998;273:11596-604.
25. Zhang H, Schaefer A, Wang Y, et al. Gain-of-Function RHOA Mutations Promote Focal Adhesion Kinase Activation and Dependency in Diffuse Gastric Cancer. Cancer Discov 2019. [Epub ahead of print].
26. Nishizawa T, Nakano K, Harada A, et al. DGC-specific RHOA mutations maintained cancer cell survival and promoted cell migration via ROCK inactivation. Oncotarget 2018;9:23198-207.
27. Ushiku T, Ishikawa S, Kakiuchi M, et al. RHOA mutation in diffuse-type gastric cancer: a comparative clinicopathology analysis of 87 cases. Gastric Cancer 2016;19:403-11.
28. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687-97.
29. Bang Y, Chung H, Xu J, et al. Pathological features of advanced gastric cancer (GC): Relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial. J Clin Oncol 2009;27:abstr 4556.
30. Maeda M, Ushijima T. RHOA mutation may be associated with diffuse-type gastric cancer progression, but is it gain or loss? Gastric Cancer 2016;19:326-8.

doi: 10.21037/dmr.2020.01.03
Cite this article as: Hao T, Jiang J, Wu W, Li M, Li L, Zhang C, He Y. *RhoA* mutations in diffuse-type gastric cancer. Dig Med Res 2020;3:4.