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EDITORIAL

RHOA mutation may be associated with diffuse-type gastric cancer progression, but is it gain or loss?

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Abbreviations

DGC	Diffuse-type gastric cancer
RHOA	Ras homolog gene family member A
RHOA-wt	*RHOA* wild type
TCGA	The Cancer Genome Atlas

RHOA, Ras homolog gene family member A, encoding a small GTPase, is a novel and contentious cancer driver gene. In 2014, frequent somatic *RHOA* mutation was identified almost exclusively in diffuse-type gastric cancer (DGC) in three studies [1–3]. Kakiuchi et al. [1] and The Cancer Genome Atlas (TCGA) Research Network [2] indicated gain-of-function mutations of *RHOA*. On the other hand, Wang et al. [3] indicated loss-of-function mutations of *RHOA*. This potential discrepancy attracted great attention from many investigators, including those who enthusiastically focus on RHOA as a potential therapeutic target. Historically, although increased RHOA expression was frequently observed in various cancers and was suggested to be associated with disease progression and poor prognosis, *RHOA* mutation was uncommon [4]. In this issue of *Gastric Cancer*, Ushiku et al. [5] report clinicopathological characteristics of *RHOA*-mutated DGCs for the first time [5].

Ushiku et al. analyzed 87 DGCs, 22 *RHOA*-mutated DGCs and 65 *RHOA* wild-type (*RHOA*-wt) DGCs, for their histopathological and clinical characteristics, especially focusing on heterogeneity of histological features and growth patterns of DGCs. *RHOA*-mutated DGCs more frequently contained focal tubular differentiation in their intramucosal areas than *RHOA*-wt DGCs. Notably, *RHOA*-mutated DGCs were significantly inclined to spread laterally in the mucosae with infiltration between normal gastric glands without well-circumscribed margins (permeative growth), resulting in a lower ratio of deeply invasive components to mucosal components in *RHOA*-mutated DGCs than in *RHOA*-wt DGCs. In previous studies [1, 3], *RHOA* mutations were detected both in intramucosal regions and in invasive regions and also both in diffuse components and in tubular components, and were considered to occur in the early phase of carcinogenesis. From these findings taken together, it was suggested that intestinal-type intramucosal gastric cancer possibly develops into DGC through stepwise progression in the presence of a *RHOA* mutation.

Ushiku et al. also investigated the clinical impact of *RHOA* mutation on patient survival, but they did not observe significant differences. However, they discuss the possibility that RHOA deregulation could be still significantly associated with patient survival for the following reasons. First, this study consisted of a small cohort; therefore, a larger cohort would be required to determine the prognostic significance of *RHOA* mutation. Second, the heterogeneity in the cohort might have influenced the result of the analysis. The cohort included five patients with

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linitis plastica type cancer (so-called scirrhous cancer), none of whom had an *RHOA* mutation. Linitis plastica, a subtype of DGC with extremely poor prognosis, might have resulted in worse prognosis of the patients without *RHOA* mutation. Third, deregulation of RHOA signaling may be caused by genomic abnormalities other than *RHOA* mutation. Indeed, *CLDN18–ARHGAP* fusion was recently identified in the genomically stable subgroup corresponding to DGC in terms of histological features. *CLDN18–ARHGAP* fusion was mutually exclusive with *RHOA* mutation and could affect the activation of RHOA-related signaling pathways [2].

Despite these novel findings, the major question of whether the *RHOA* mutation is a gain-of-function or a loss-of-function mutation still remains unsolved. The three initial studies conducted whole-exon and/or whole-genome sequencing, and reported that frequent *RHOA* mutation was observed almost exclusively in DGC or the genomically stable subgroup (14.3–25.3 %, Table 1) [1–3]. Most *RHOA* mutations were unevenly distributed in the amino-terminal domain and weakly clustered in two adjacent functional domains involved in GTP binding and interaction with RHOA effectors. The most frequent mutations were R5Q and Y42C, followed by G17E, in Kakiuchi et al. [1]; Y42C followed by L57V in Wang et al. [3]; and Y42C followed by Y42S in TCGA [2]. These sites and regions were referred to as hotspots and hotspot regions (HS1/2) [1–3]. Kakiuchi et al. stated that mutational hotspot residues in their study (R5, Y42, and G17) were highly conserved among Rho family proteins. The presence of such hotspot regions suggests a gain-of-function mutation. At the same time, *RHOA* mutations often conformed to the classic two-hit suppressor gene model, being associated with concurrent loss of heterozygosity, which suggested a loss-of-function mutation [3].

Biological analysis revealed multiple aspects of *RHOA* mutations. Wang et al. [3] found that *RHOA* mutants

Table 1 The comparison of three studies on *RHOA* mutation

Authors	Subjects and classification)	Method	Frequency of *RHOA* mutation	Mutation sites[a]	Additional findings	Effect of mutants, and type of cell/analysis	Suggested mutation type
Kakiuchi et al. [1]	Diffuse-type gastric cancer, 30 cases for exploration and 57 cases for validation; intestinal-type gastric cancer, 51 cases (Lauren classification)	WES	Diffuse type, 25.3 % (22/87); intestinal type, 0 % (0/51)	Hotspot, R5W (5), G17E (3), Y42C (6); others, R5Q (1), L22R (1), V38G (1), E54K (1), W58S (1), R68P (1), L69R (2), Y74D (1)		Growth-promoting effect (SW948 cell line)	Gain of function
Wang et al. [3]	Gastric cancer, 100 cases for exploration and 183 cases for validation (WHO classification)	WGS	Diffuse type, 14.3 % (14/98); mixed/indeterminate type, 7.8 % (4/51); intestinal type, 0 % (0/134)	Hotspot, Y42C (8), L57V (4); others, R5W (2), G17E (2), L22R (1), Y34C (1), F39V (1), E40V (1), D59G (1), A61D (1), G62E (1), L81fs[b]	Concurrent LOH (L57V, Y42C, E40V, D59G), two-hit mutations	(1) Impairment of GTP binding capacity (293T/17 cells), (2) escape from anoikis (mouse intestinal organoids)	Loss of function
TCGA Research Network [2]	Gastric cancer, 295 cases	WES/WGS	Whole cohort, 5.4 % (16/295); GS, 15 % (9/58)	Hotspot region HS1 (effector region), Y42C (3), Y42S (2), Y34C (1), F39C (1), E40K (1), N41K (1); hotspot region HS2 (G box binding region). L57V (1), D59Y (1), T60K (1), A61D (1), G62R (1), G62E (1); others, R5W (1), G17E (1)	*CLDN18–ARHGAP* fusion (mutually exclusive with *RHOA* mutation)	Activation of RhoA-ROCK pathway (bioinformatic analysis through evaluation of gene expression status)	Gain of function

In the study by The Cancer Genome Atlas (*TCGA*) Research Network, gastric cancers were divided into four groups on the basis of the comprehensive molecular approach. Diffuse-type gastric cancer was enriched in the genomically stable (*GS*) subtype (40/55).

LOH loss of heterozygosity, *ROCK* Rho-associated protein kinase, *WES* whole-exon sequencing, *WGS* whole-genome sequencing

[a] The number of mutations is given in *parentheses*.

[b] *fs* indicates a truncating alteration.

(Y42C, L57V) promoted escape from anoikis in mouse intestinal organoid cultures, which might be involved in the diffuse morphological phenotype. Kakiuchi et al. [1] demonstrated a growth-promoting effect of *RHOA* mutants (G17E, Y42C) in an *RHOA*-mutant cell line (SW948). The fact that *RHOA* mutants could show oncogenic phenotypes in the presence of a wild-type allele suggested a gain-of-function effect of *RHOA* mutation. Additionally, bioinformatic analysis predicted activation of the RhoA–Rho-associated protein kinase signaling pathway through evaluation of gene expression status in the pathways putatively regulated by *RHOA* [2]. In contrast, biochemical analysis showed that *RHOA* mutants (Y42C, L57V) had a significantly reduced amount of the GTP-bound active form compared with *RHOA*-wt in 293T/17 cells (a derivative of 293T cells which are isolated from human embryonic kidneys). The defective RHOA activity showed a loss-of-function effect of the *RHOA* mutations [3].

These data showed the hotspot *RHOA* mutants had characteristics of both "biochemical loss of function" and "biological gain of function." Namely, the hotspot *RHOA* mutants not only lost their GTP-binding capacity but also acquired an oncogenic activity, possibly in an unidentified signaling pathway. A remarkable example of such an acquisition of oncogenic activity unrelated to intrinsic physiological activity is known for *IDH1* and *IDH2* genes, which are recurrently mutated in glioma [6, 7] and acute myeloid leukemias[8]. *IDH1*/*IHD2* mutants acquire a neomorphic enzymatic ability to convert α-ketoglutarate into 2-hydroxyglutarate [9], which is unrelated to its physiological activity to mediate the reversible conversion between isocitrate and α-ketoglutarate and causes suppression of epigenetic enzymes, TET enzymes, resulting in aberrant epigenetic and gene expression profiles [10]. Neomorphic activity of *RHOA* mutants may well explain the biochemical loss of function and biological gain of function. The clinicopathological behaviors of DGCs with *RHOA* mutation reported here may lead to a clue.

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