Pathol. Oncol. Res. (2015) 21:181–185
DOI 10.1007/s12253-014-9804-8

RESEARCH

Frameshift Mutations of Cadherin Genes *DCHS2*, *CDH10* and *CDH24* Genes in Gastric and Colorectal Cancers with High Microsatellite Instability

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Received: 19 November 2013 / Accepted: 22 May 2014 / Published online: 5 June 2014

Abstract Cadherins (CDHs) are important in maintenance of cell adhesion and polarity, alterations of which contribute to tumorigenesis. Alterations of E-cadherin, a prototype CDH, have been reported in many cancers. However, alterations of unconventional CDHs, including CDH10, CDH24 and DCHS2 are largely unknown in cancers. Aim of this study was to explore whether *CDH10*, *CDH24* and *DCHS2* genes are mutated in gastric (GC) and colorectal cancers (CRC). In a public database, we found that *CDH10, CDH24* and *DCHS2* genes had mononucleotide repeats in the coding sequences that might be mutation targets in the cancers with microsatellite instability (MSI). We analyzed the mutations in 89 GC and 131 CRC (high MSI (MSI-H) or stable MSI/low MSI (MSS/MSI-L)) by single-strand conformation polymorphism analysis and DNA sequencing. We found six *DCHS2*, one *CDH10* and one *CDH24* frameshift mutations in them. All of the mutations were detected in cancers with MSI-H and there was a statistical difference in the frameshift mutation frequencies between the cancers with MSI-H (8/105) and MSS/MSI-L (0/115). The *DCHS2* frameshift mutations were found in 8.8 % and 4.2 % of GC and CRC with MSI-H respectively. Our results show that unconventional *CDH10, CDH24* and *DCHS2* genes harbored frameshift mutations. These mutations might inactivate the cell adhesion-related functions and could be a feature of GC and CRC with MSI-H.

Keywords DHCS2 · CDH10 · CDH24 · Cadherin · Mutation · Cancer · Microsatellite instability

Introduction

Cadherins (CDHs) are calcium-dependent adhesion-related transmembrane proteins. They play crucial roles in cell adhesion, forming adherens junctions to bind cells together [1–3]. Each CDH has a cytoplasmic component, a transmembrane component, and an extracellular component. To date, there are over 80 types of CDH in humans, which can be classified into four groups: classical, desmosomal, protocadherins and unconventional [1, 2]. CDHs are involved in many biological processes, including cell adhesion, tissue development, cytoskeletal organisation and cell migration, as well as in diseases such as cancers [1–3]. CDHs maintain cellular membrane structures and hence disruption of the E-cadherin (a classical CDH) –catenin complex causes loss of cell adhesion [4]. CDH-mediated cell adhesion also participates in transduction of transmembrane signal, which regulates gene expression and cell fate [3, 4]. In general, loss of E-cadherin function by loss of expression and mutation are observed in many cancers and associated with more aggressive tumors and poor prognosis [5–8]. Although normal functions and alterations of the classical CDHs have been studied well in many cancers [1, 2], those of unconventional CDHs, including CDH10, CDH24 and DCHS2 (also known as cadherin J), are largely unknown. Expression of CDH10 in brain endothelium appears to maintain blood brain barrier [9]. The CDH10 is expressed in normal prostate cells, but is lost in prostate cancers [10]. CDH24 is ubiquitously expressed in many tissues and mediates strong cell-cell adhesion, but its function in cancer is not reported [11]. DCHS2 is expressed in cerebral cortex and testis and is a potential

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candidate for affecting Alzheimer's disease [12], but its function in cancer is not reported, either.

In a public genome database (http://genome.cse.ucsc.edu/), we found that *CDH10, CDH24* and *DCHS2* genes have mononucleotide repeats in their coding sequences that could be targets for frameshift mutation in cancers with microsatellite instability (MSI). Frameshift mutations of genes with mononucleotide repeats are features of gastric (GC) and CRC with microsatellite instability (MSI) [13]. Frameshift mutations in *CDH10, CDH24* and *DCHS2* might cause alterations of their functions and contribute to cancer pathogenesis. However, the data on mutations in *CDH10, CDH24* and *DCHS2* genes are known neither in GC nor CRC. In this study, we analyzed the mononucleotide repeats in *CDH10, CDH24* and *DCHS2* and found that both genes were mutated in GC and CRC with MSI.

Materials and Methods

Tissue Samples and Micro Dissection

For the mutation analysis, methacarn-fixed tissues of sporadic 89 GC and 131 CRC were used in this study. All of the patients with the cancers were Koreans. The GC consisted of 34 GC with high MSI (MSI-H), 55 GC with stable MSI/low MSI (MSS/MSI-L), 71 CRC with MSI-H and 60 CRC with MSS/MSI-L. The MSI evaluation system used five mononucleotide repeats (BAT25, BAT26, NR-21, NR-24 and MONO-27), tumoral MSI status of which was characterized as: MSI-H, if three or more of these markers show instability, MSI-L, if one or two of the markers shows instability and MSS, if none of the markers shows instability [14]. The pathologic features of the cancers are summarized in Table 1. The histologic features of CRC with MSI-H, including mucinous histology, tumor infiltrating lymphocytes, medullary pattern, and Crohn's like inflammation, were evaluated in all blocks of all cases by a pathologist. Malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle by microdissection as described previously [15, 16]. DNA extraction was performed by a modified single-step DNA extraction method by proteinase K treatment. Approval of this study was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study.

Single strand conformation polymorphism (SSCP) analysis

CDH10 exon 12 (A7 repeat), *CDH24* exon 5 (G7) and *DCHS2* exon 7 (A8 repeat) have mononucleotide repeats in their coding sequences. Genomic DNA from the microdissected cells was isolated, and was amplified by polymerase chain reaction (PCR) with specific primer pairs. Radioisotope ([^{32}P] dCTP) was incorporated into the PCR products for detection by autoradiogram. After SSCP, mobility shifts on the SSCP gels (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) were determined by visual inspection. Direct DNA sequencing reactions in both forward and reverse sequences were performed in the cancers with the mobility shifts in the SSCP using a capillary automatic sequencer (3730 DNA Analyzer, Applied Biosystem, Carlsbad, CA, USA). When mutations in the genes were suspected by SSCP, analysis of an independently isolated DNA from another tissue section of the same patients was performed to exclude potential artifacts originated from PCR. Other procedures for PCR-SSCP were described in our previous reports [15–17].

Results

Genomic DNAs isolated from normal and tumor tissues of the 89 GC and 131 CRC were analyzed for detection of mutation

Table 1 Summary of pathologic features of the cancers

No. of gastric carcinomas				No. of colorectal carcinomas			
		MSI-H (n=34)	MSS/MSI-L (n=55)			MSI-H (n=71)	MSS/MSI-L (n=60)
TNM	I	13	18	TNM	I	15	9
	II	13	22		II	26	21
	III	7	12		III	27	29
	IV	1	3		IV	3	3
Lauren's subtype	Diffuse	20	30	Location (colon)	Cecum	16	0
	Intestinal	14	25		Ascending	44	3
					Transverse	9	3
EGC Vs. AGC	EGC	3	4		Descending & sigmoid	2	21
	AGC	31	51		Rectum	0	33

EGC early gastric cancer, *AGC* advanced gastric cancer, *TNM*, tumor, lymph node, metastasis

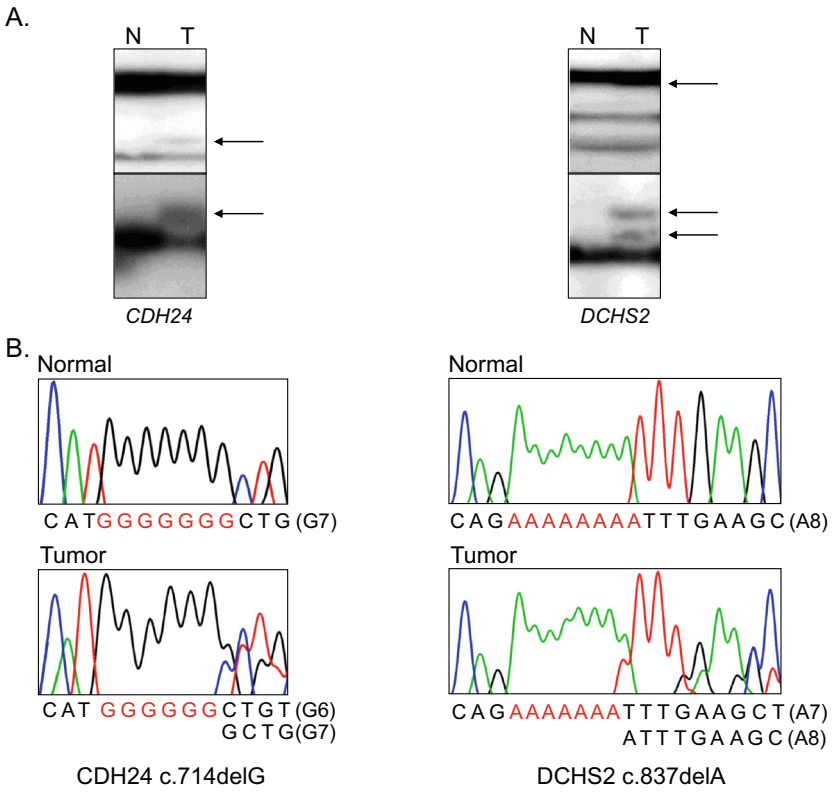

Fig. 1 Representative SSCP and DNA sequencing of *CDH24* and *DCHS2* genes SSCP **a** and DNA sequencing analyses **b** of *CDH24* (left) and *DCHS2* (right) from tumor (Lane T) and normal tissues (Lane N). **a** In the SSCP, the arrows (Lane T) indicate aberrant bands compared to the SSCP from normal tissues (N). **b** Direct DNA sequencing analyses of the PCR product of *CDH24* (left) and *DCHS2* (right) show heterozygous deletions of a nucleotide in tumor tissue as compare to normal tissues

in *CDH10* (A7), *CDH24* (G7) and *DCHS2* (A8 repeat) by PCR-SSCP analysis. On the SSCP, we found aberrantly migrating bands in six cases of *DCHS2,* one case of *CDH10* and one case of *CDH24* (Fig. 1 and Table 2). DNA from the normal tissues from corresponding patients showed no evidence of aberrant migration on SSCP, indicating the mutations had risen somatically (Fig. 1a). All of the mutations were interpreted as heterozygous according to the SSCP and direct sequencing analyses (Fig. 1a and b). Direct DNA sequencing of the cancer tissues with the aberrant bands on SSCP confirmed that they represented somatic mutations of *CDH10, CDH24* and *DCHS2* genes (Fig. 1b). All of the mutations were deletion mutations in the repeats that would cause premature stops, which would lead to termination of translation (Table 2). Three of 34 GC (8.8 %) and three of 71 CRC (4.2 %) with MSI-H harbored *DCHS2* frameshift mutations, while one of 34 GC (2.9 %) and one of 71 CRC (1.4 %) with MSI-H harbored *CDH10* and *CDH24* mutation, respectively.

All of the mutations were detected in cancers with MSI-H, but not in those with MSS/MSI-L (Table 2). There was a statistical difference in the frameshift mutation frequencies between the cancers with MSI-H (8/105) and MSS/MSI-L (0/115) (Fisher's exact test, $p=0.002$). In terms of tissue origins, there was no statistical difference in prevalence of the mutations between GC and CRC (Fisher's exact test, $p>0.05$). There was no significant association of the mutations with the clinicopathologic data of the patient (age, sex, histologic grade and stage). In the cancers with MSI-H, there was no correlation between histological features of the tumors (histologic grade, subtypes, mucinous histology, medullary pattern and tumor-infiltrating lymphocytes) and presence of the mutations.

Table 2 Summary of *CDH10, CDH24* and *DCHS2* mutations in gastric and colorectal cancers

Gene	Location	Wild type	Mutation	MSI status of the mutation cases (n)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
CDH10	Exon 12	A7	A6	MSI-H (1)	Gastric : 1/34 (2.9)	c.2054delA (p. Lys685Serfsx34)
CDH24	Exon 5	G7	G6	MSI-H (1)	Colorectal: 1/71 (1.4)	c.714delG (p. Leu239Cysfsx7)
DCHS2	Exon 7	A8	A7	MSI-H (6)	Gastric: 3/34 (8.8) Colorectal: 3/71 (4.2)	c.837delA (p. Lys279Asnfsx10)

Discussion

It is now well known that epithelial-mesenchymal transition (EMT) is actively involved in cancer progression as well as cancer development [4]. EMT allows polarized epithelial cells to undergo changes that enable them to possess a mesenchymal cell phenotype [4]. One of the most crucial phenotypes is alteration of cytoskeletal proteins, including loss of E-cadherin and cytokeratin expression, and gain of N-cadherin, osteoblast-cadherin and β-catenin [4]. Cells that lack E-cadherin show increased tumorigenicity and metastasis when transferred.

into immunodeficient mice [18]. Despite the importance, however, somatic mutation status of cadherin-encoding genes remains elusive in cancers. In the present study, we attempted to disclose whether somatic frameshift mutations of *CDH10, CDH24* and *DCHS2* were present in GC and CRC. Since mononucleotide repeats are common targets for somatic mutations in cancers with MSI-H, we focused the analysis within the repeats in *CDH10, CDH24* and *DCHS2* genes. We found that 8.8 % of GC and 4.2 % of CRC with MSI-H harbored *DCHS2* frameshift mutations, while 2.9 % of GC and 1.4 % of CRC with MSI-H harbored *CDH10* and *CDH24* mutations, respectively. Of note, there was a significant difference of the mutation frequencies between the cancers with MSI-H and MSS/MSI-L, indicating that the cadherin gene mutations with MSI-H were specific. These results indicate that *DCHS2* gene is frequently altered in GC and CRC with MSI-H by somatic frameshift mutation that might alter the function of DCHS2 protein.

In the present study, we found three types of mutations (Table 2), which would delete amino acids after the frameshift mutations and hence would resemble a typical loss-of-function mutation. The *DCHS2* mutation would alter or delete amino acids after the 279th residue (Table 2). Because the full-length DCHS2 protein is 2916 amino acids long, the mutant DCHS2 would lose over 90 % of the amino acids that may important proper functions of the protein. However, it is not possible to guess consequences of the *DCHS2* mutation, because to our knowledge there is no data on DCHS2 functions in relation to tumorigenesis. In the present study, we provide evidence that shows cancer-related alterations of *DCHS2* gene. This information may provide basis for further efforts to elucidate its roles in physiological and pathological status. In the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic) that catalogues somatic mutations in cancer, we found *DCHS2* somatic mutations in many types of cancers, including colon, prostate, lung and kidney cancers [19–21]. These data suggest a possibility that *DCHS2* alterations might be causally related to cancer pathogenesis. As for CDH10, its loss in luminal prostate cells is evident in prostate cancers, indicating its specific role in secretory cell terminal differentiation in prostate cancers [10]. Similarly, it is also possible to hypothesize that inactivation of CDH10 plays a role in GC and CRC development. CDH24 has an ability to bind with α-catenin, β-catenin and p120 catenin, and mediates cell adhesion [11]. Provided that CDH10 and CDH24 maintain normal cell adhesion and polarity, inactivation of them by the frameshift mutation appears to contribute to development of cancers.

In summary, we found frameshift mutations of *DCHS2, CDH10 and CDH24* that may inactivate their functions and might possibly alter EMT in the affected GC and CRC. However, it remains to be elucidated whether the mutant DCHS2, CDH10 and CDH24 promote tumorigenesis, whether they are involved in EMT and whether loss of the functions are related to MSI-H phenotypes.

Acknowledgments This study was supported by a grant from National Research Foundation of Korea (2012R1A5A2047939 and 2012R1A1B3000458).

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