Down-regulation of *drs* mRNA in Colorectal Neoplasms

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The *drs* gene was originally isolated as a transformation suppressor gene against the *v-src* oncogene. Expression of *drs* mRNA is down-regulated by retroviral oncogenes such as *v-src* and *v-K-ras* in the rat cell line F2408. Expression of *drs* mRNA is also markedly reduced in a variety of human cancer cell lines, including those of carcinomas of the colon, bladder, and ovary, suggesting that down-regulation of *drs* mRNA is correlated with the development of human cancers. To clarify the correlation between down-regulation of the *drs* gene and malignant tumor formation in human colorectal neoplasms, we examined expression of *drs* mRNA in a variety of colon cancer tissues by *in situ* hybridization. A total of 53 morphologically distinct neoplastic specimens were divided into the following five groups according to morphology: low and high grade adenoma in 7 and 12 cases, respectively (groups A, B), protruded-type carcinoma in 16 (group C), superficial-type carcinoma with an adenomatous component in 10 (group D) or superficial-type carcinomas without any adenomatous component in 8 (group E). Expression of *drs* mRNA was detected in normal mucosa, low-grade adenoma and most superficial-type carcinomas without any adenomatous component.

On the other hand, the rate of *drs* mRNA expression was significantly lower in protruded-type adenocarcinoma and superficial-type carcinoma with an adenomatous component. Our results indicate that down-regulation of *drs* mRNA is closely correlated with carcinomas which arise from adenomatous polyps in the course of the adenoma-carcinoma sequence, but that most carcinomas arising *de novo* are independent of the tumor suppressor function of the *drs* gene.

Key words: Colorectal carcinoma — *drs* gene — Adenoma-carcinoma sequence — *de novo* carcinoma-genesis

Human oncogenesis involves multistep genetic alterations, including activation of dominant oncogenes and inactivation of tumor-suppressor genes. The *drs* gene was isolated as a suppressor gene for *v-src* transformation from a cDNA library of primary rat embryo fibroblasts. The *drs* gene is also down-regulated by other retroviral oncogenes, such as *v-fps*, *v-ras*, *v-mos*, *v-sis*, and *v-abl*, but not by large T antigen of simian virus 40 (SV40) or by *v-onco-K-ras* in the rat cell line F2408. Expression of *h-drs* mRNA was detected in normal mucosa, low-grade adenoma and most superficial-type carcinomas without any adenomatous component.

Expression of *h-drs* mRNA in a variety of human colorectal carcinoma tissues by *in situ* hybridization is closely correlated with development of colorectal carcinoma, namely the adenoma-carcinoma sequence, and *de novo* carcinogenesis. The former, proposed by Morson, claims that almost all colorectal carcinomas arise from adenomatous polyps. In contrast, the latter is a nonpolypoid growth theory proposed by Helwig and others. To clarify the role of the *drs* gene in these two distinct colorectal carcinogenesis theories, we examined the expression of *drs* mRNA in normal colorectal mucosa and various neoplasms by *in situ* hybridization.

**MATERIALS AND METHODS**

**Materials** We used 19 cases of colonic adenomas and 34 cases of intramucosal and submucosally invasive colonic carcinomas. These tumors had been resected in our affiliated hospitals in Shiga and Kyoto prefectures, Japan, between 1995 and 2001. All tissues were fixed in 10% formalin for 24–48 h, and embedded in paraffin. Each paraffin block, including the maximum diameter of the tumor, was thinly sliced into serial sections.

**Histological studies** We classified the neoplasms using hematoxylin and eosin-stained sections into the following five groups according to morphology: group A (low-grade adenoma) in 7 cases, group B (high-grade adenoma) in 12,
group C (protruding-type carcinoma) in 16, group D (superficial-type carcinoma with an adenomatous component) in 10 and group E (without any adenomatous component) in 8. The superficial-type carcinomas were defined according to the following criteria: protruding portion of the carcinoma lesion was less than twice the height of the normal mucosa, or the lesion height was smaller than the maximum diameter of the lesion. Therefore, superficial-type carcinomas in this study included both the depressed and flat-type carcinomas. The diagnoses of adenoma/dysplasia and carcinoma corresponded to the Vienna classification of gastrointestinal epithelial neoplasia.19 Groups A and B were composed of neoplasia in categories 3 and 4.1 in the Vienna classification, respectively. On the other hand, group C, group D and group E were composed of neoplasia in categories 4.2 to 5.2. Morphological evidence indicates that colorectal carcinomas may arise either from adenomatous polyps in the course of the adenoma-carcinoma sequence, or carcinomas arising de novo. We defined the morphological features of de novo carcinomas as (i) appearance as flat elevations with or without a central depression, (ii) invasive growth that occurs before they reach the diameter of 1 cm, (iii) lack of any adenomatous component,20 (iv) limited to the mucosa (intramucosal carcinoma) or involves only the submucosal layer.

**In situ hybridization** As the probe, human drs cDNA was cloned into the plasmid pBluescriptIISK (−). Anti-sense and sense RNA probes were labeled with digoxigenin-11-UTP by *in vitro* transcription using a commercial kit (Roche-Boehringer-Mannheim, Indianapolis, IN). Labeled riboprobes were then fragmented to about 100 bp in length by alkaline hydrolysis. The sense probe served as the negative control. For *in situ* hybridization, a streptavidin-biotin technique was used (DAKO, LSAB-2 kit, Carpinteria, CA). Peroxidase binding sites were visualized by the diaminobenzidine method and the nuclei were lightly counter-stained with hematoxylin.

**RESULTS**

To clarify the role of the drs gene in the development of colorectal carcinoma, *in situ* hybridization was performed on 19 adenomas and 34 adenocarcinomas using anti-sense probes. Table I. Down-regulation of drs mRNA in Each Group

| Group | Expression of drs mRNA | Total |
|-------|------------------------|-------|
|       | (−) | (+) |
| Group A | 0 | 7 (100%) | 7 |
| Group B | 8 (66.7%) | 4 (33.3%) | 12 |
| Group C | 14 (87.5%) | 2 (12.5%) | 16 |
| Group D | 7 (70.0%) | 3 (30.0%) | 10 |
| Group E | 2 (25.0%) | 6 (75.0%) | 8 |

Group A, low-grade adenoma; group B, high-grade adenoma; group C, protruded-type carcinoma; group D, superficial-type carcinoma with adenomatous component; group E, superficial-type carcinomas without any adenomatous component.

Fig. 1. *In situ* hybridization of low-grade adenoma with sense probe (C, E) or anti-sense probe (A, B, D). (A) Low-power view of low-grade adenoma and normal mucosa. In normal mucosa and low-grade adenoma, marked expression of drs mRNA is evident in the cytoplasm of glandular cells. (B, C) High-power view of the normal mucosa in A. (D, E) High-power view of the low-grade adenoma in A.
and sense riboprobes of human *drs* cDNA. The *drs* antisense probe specifically and efficiently detected expression of *drs* mRNA. The results of *in situ* hybridization on the normal mucosa and colorectal neoplasms are summarized in Table I. All normal mucosa and low-grade adenomas exhibited strong expression of *drs* mRNA, and *drs* mRNA was detected in stromal inflammatory cells (Fig. 1). In contrast, the negative rate of *drs* mRNA was 8/12 (67.7%) in group B, 14/16 (87.5%) in group C and 7/10 (70.0%) in group D (Figs. 2, 3). Our results indicate that expression of the *drs* gene is down-regulated in the progression from adenoma to adenocarcinoma, suggesting a tumor-suppressor function of the *drs* gene in the genesis of colorectal adenocarcinoma. On the other hand, *drs* mRNA was significantly expressed in most superficial-type carcinomas without any adenomatous component (group E) (Fig. 4). These present results suggest some carcinomas in group C and group D and most carcinomas in group E are independent of down-regulation of the *drs* gene.

**DISCUSSION**

Large discrepancies between Western and Japanese pathologists exist in the diagnosis of adenoma/dysplasia versus carcinoma for colorectal glandular lesions. The concept and definition of intramucosal carcinoma is used...
by Japanese pathologists, whereas Western pathologists use the term dysplasia. This issue was addressed when the category of intramucosal carcinoma was accepted for early lesions of advanced carcinoma in the Vienna classification. In the present study, to resolve these large differences among pathologists, we used the Vienna classification of gastrointestinal epithelial neoplasia, which is based on cytological and architectural severity and invasion status. In colon carcinogenesis, the theory of the adenoma-carcinoma sequence with accumulative genetic mutations in the order of \( APC \), \( K\text{-}ras \), \( p53 \), and \( DCC \), is generally well accepted. One of the cornerstones of this proposal is the model represented by familial adenomas, which result from initial mutations of the \( APC \) gene, followed by occurrence of \( K\text{-}ras \) gene mutation, and progression into carcinoma, where cumulative mutations of \( p53 \) and \( DCC \) occur. It has been surmised that this same mechanism also applies to sporadic colorectal carcinogenesis. In contrast, the frequency of \( APC \) alterations in superficial depressed tumors has been shown to be significantly lower than that in protruding ones, and mutant \( K\text{-}ras \) is associated with polyoid growth carcinomas rather than non-polyoid growth carcinomas. The superficial-type colorectal tumors are etiologically distinct from ordinary colorectal polyoid tumors and therefore may follow an alternative colorectal tumorigenesis pathway.

Inoue et al. have previously reported that \( drs \) has the ability to suppress \( v\text{-}src \) and \( v\text{-}K\text{-}ras \) transformation without disturbing cell proliferation in the rat cell line F2408, and Yamashita et al. have reported that induction of the \( drs \) gene in colon cancer cell lines causes suppression of anchorage-independent growth of these cells. To elucidate the role of the \( drs \) gene in distinct colorectal carcinogenesis, we examined expression of \( drs \) mRNA in normal mucosa, various types of adenomas and morphologically distinct adenocarcinomas by \textit{in situ} hybridization. \( drs \) mRNA was expressed in all normal mucosa and low-grade dysplasia, whereas \( drs \) mRNA expression was significantly lower in high-grade adenoma, protruded-type adenocarcinoma and superficial-type carcinoma with an adenomatous component. Our results suggest that expression of the \( drs \) gene is down-regulated in the progression from adenoma to adenocarcinoma, suggesting a tumor-suppressor function of the \( drs \) gene in the genesis of colorectal adenocarcinoma. The present results are consistent with our previous results on human colon adenocarcinoma cell lines and colorectal tissues, supporting the conclusion that down-regulation of \( drs \) mRNA plays a crucial role in the development of polyoid-growth adenocarcinoma. The simplest explanation for the reduced expression of \( drs \) mRNA in polyoid growth colorectal carcinomas is that expression of \( drs \) mRNA is down-regulated by activation of endogenous proto-oncogenes. Indeed, activation of the endogenous proto-oncogenes \( ras \) and \( src \) has been reported for colorectal cancers.

In the present study, significant \( drs \) mRNA expression was observed in 2 of 16 cases of polyoid growth carcinoma. One of them had no adenomatous remnants. Umetani et al. reported that a superficial early carcinoma may become a polyoid-type proberant advanced carcinoma. This polyoid-growth carcinoma that exhibited strong expression of \( drs \) mRNA may be an exophytic-growth \textit{de novo} carcinoma. On the other hand, significant \( drs \) mRNA expression was observed in most superficial carcinomas without any adenomatous component. These results suggest that the carcinogenesis pathway of \textit{de novo} carcinomas is independent of down-regulation of the \( drs \) gene, and may arise via a different route of malignant transformation. Minamoto et al. reported that superficial-type adenocarcinomas classified as \textit{de novo} carcinomas may arise from very small superficial-type adenomas. In the present study, \( drs \) mRNA expression was down-regulated in 2 of 8 superficial carcinomas without any adeno-

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**Fig. 4.** \textit{In situ} hybridization of superficial-type carcinomas without any adenomatous component with sense probe (C) or anti-sense probe (A, B). (A) Low-power view of superficial-type carcinomas and normal mucosa. Marked expression of \( drs \) mRNA was evident in carcinoma cells and normal mucosa. (B) High-power view of panel A. (C) Serial section of panel B.
matous component. These cases may be carcinomas arising from flat adenoma in the course of the adenoma-carcinoma sequence.

In conclusion, colorectal neoplasms may develop along different genetic pathways that may determine the morphological variations observed in the human colon. Down-regulation of drs mRNA is significantly associated with carcinoma which may arise from adenomatous polyps in the course of the adenoma-carcinoma sequence. Most carcinomas arising de novo are independent of down-regulation of the drs gene.

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