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ORIGINAL ARTICLE

Differential expression of nucleolin in colon adenoma and adenocarcinoma

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Abstract

Objective The aim of the study was to investigate the discrepancy in nucleolin expression between colon adenoma and colon adenocarcinoma, explore the role of nucleolin expression in the carcinogenesis of colon adenocarcinoma, and determine the correlation of the nucleolin expression level with histological grade in colon adenocarcinoma.

Methods In total, 80 cases of colon adenocarcinoma with cancer-adjacent colon mucosa and 60 cases of colon adenomas were examined by immunohistochemistry using an antibody against nucleolin. Nucleolin expression levels in these groups were compared. The correlation between the nucleolin expression level and grade of colon adenocarcinoma was analyzed.

Results Nucleolin expression is located in the nuclei of colon adenocarcinoma, colon adenoma, and cancer-adjacent colon mucosa tissues with different intensities. A semiquantitative evaluation using the Allred scoring system showed that the nucleolin immunostaining score in colon adenocarcinoma (7.8 ± 0.1) was significantly higher than those in colon adenoma (6.3 ± 0.2) and cancer-adjacent colon mucosa (5.4 ± 0.1; $P < 0.01$). The nucleolin immunostaining score in colon adenoma was significantly higher than that in cancer-adjacent colon mucosa ($P < 0.01$). Nucleolin expression levels in well-differentiated and moderately differentiated adenocarcinoma (6.8 ± 0.2) were significantly lower than those in poorly differentiated adenocarcinoma (8.0 ± 0.1; $P < 0.01$).

Conclusion Increased nucleolin expression may play an important role in the process of malignant transformation of colon adenocarcinoma and predicts a poor prognosis.

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Key words: nucleolin; colon adenoma; colon adenocarcinoma

Colon adenoma is considered a precursor lesion of colon adenocarcinoma. The protein changes driving this process are complex. Understanding the mechanisms of adenoma-to-carcinoma transformation is critical to gain insight into the triggers of invasion and proliferation of the cancer.

Nucleolin is a nuclear protein with a molecular weight of 105 kD; it is abundant in the nucleolus and mainly distributed in the nucleolar organizer region [1]. Nucleolin has been implicated in many cellular processes, including the transcription, packing, and transport of ribosomal RNA, DNA replication and recombination, cell cycle progression, and apoptosis [2]. Expression of nucleolin in normal cells is limited to the nuclei, but in active cells or malignant tumor cells, it is overexpressed and translocated from the nucleus to the cytoplasm [3–5]. To date, only a few studies have examined nucleolin expression in human cancer tissues [3, 6–8], and none have examined colon adenocarcinoma as well as its precursor lesions. In the current study, alterations in nucleolin expression in colon adenoma and colon adenocarcinoma tissues as well as its prognostic impact were examined.

Materials and methods

Human tissues

Formalin-fixed paraffin-embedded tissue samples from 80 cases of colon adenocarcinoma with cancer-adjacent colon mucosa tissues and 60 cases of colon adenoma were retrieved from the archive files of Department of Pathology, School of Fundamental Medicine and Affiliated Hospital, Xiangnan University (China) from 2013 to 2015. No patient accepted any therapy before the operation.

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Immunohistochemistry

Sections were deparaffinized, rehydrated, and washed in phosphate-buffered saline (PBS, pH 7.5) for 15 minutes. Endogenous peroxidase activity was blocked by incubation for 30 minutes in a 3% hydrogen peroxide solution. Tissue antigens were retrieved by pressure cooking at 120 °C for 8 minutes in a citrate buffer solution, pH 7.0. The specimens were washed once with PBS. Non-specific binding was blocked by incubating the slides with normal goat serum in PBS for 30 minutes at 37 °C and then incubated overnight at 4 °C with the primary antibody against nucleolin (MS-3, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After sections were washed with PBS 3 times, they were incubated with the secondary antibody for 40 minutes at 37 °C. After they were washed with PBS 3 times, the sections were immunostained with avidin-biotin complex for 40 minutes at 37 °C. Visualization of the immunoreaction was conducted with 3,3'-diaminobenzidine (DAB; Sigma, Co., St. Louis, MO, USA) for 5 minutes. Finally, sections were counterstained with hematoxylin. Positive and negative controls were used for each section.

Staining was semiquantitatively examined by 2 independent pathologists using the Allred 8-unit system [9]. The positively stained tumor cells were scored as follows: 0, none; 1, < 1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, > 2/3. The staining intensity of the positive tumor cells was scored as follows: 0, none; 1, weak; 2, intermediate; and 3, strong. For each case, the staining proportion and intensity scores were determined. The differences in nucleolin expression levels among colon adenocarcinoma, colon adenoma, and cancer-adjacent colon mucosa were evaluated, and the correlation of the nucleolin expression level with the grade of colon adenocarcinoma was analyzed.

Statistical analysis

SPSS 13.0 was used for the statistical analysis. The differences in the immunostaining score among groups were evaluated using unpooled *t*-tests. Differences in percentage were evaluated using Fisher's exact tests. A *P*-value of < 0.05 was considered statistically significant.

Results

Immunohistochemically, the nuclei were positively stained for nucleolin in all tissues samples, with various staining intensities. In cancer-adjacent colon mucosa, the nuclei of glandular cells exhibited weak staining for nucleolin with low immunostaining scores of 5.4 ± 0.1 (Fig. 1). In colon adenoma, the nuclei of glandular cells exhibited moderate staining for nucleolin with an intermediate immunostaining score of 6.3 ± 0.2 (Fig. 2). In contrast, the colon adenocarcinoma was strongly positive for nucleolin, with a high immunostaining score of 7.8 ± 0.1 (Fig. 3 and 4). A statistical evaluation revealed that the nucleolin immunostaining score was significantly higher in colon adenocarcinoma than in colon adenoma and cancer-adjacent colon mucosa ($P < 0.01$). The nucleolin immunostaining score in colon adenoma was significantly higher than that in cancer-adjacent colon mucosa ($P < 0.01$). The nucleolin immunostaining scores of cancer-adjacent colon mucosa, colon adenoma, and colon adenocarcinoma were summarized in Table 1.

Moreover, when comparing the nucleolin immunostaining score in colon adenocarcinomas with different histological grades, we found that the nucleolin immunostaining score in low-grade colon adenocarcinoma (6.8 ± 0.2; Fig. 3) was significantly lower than that in high-grade colon adenocarcinoma (8.0 ± 0.1; Fig. 4; $P < 0.01$). The immunostaining scores in colon adenocarcinoma with different grades were summarized in Table 2. These data demonstrate that the nucleolin expression level is positively correlated with the histological grade of colon adenocarcinoma.

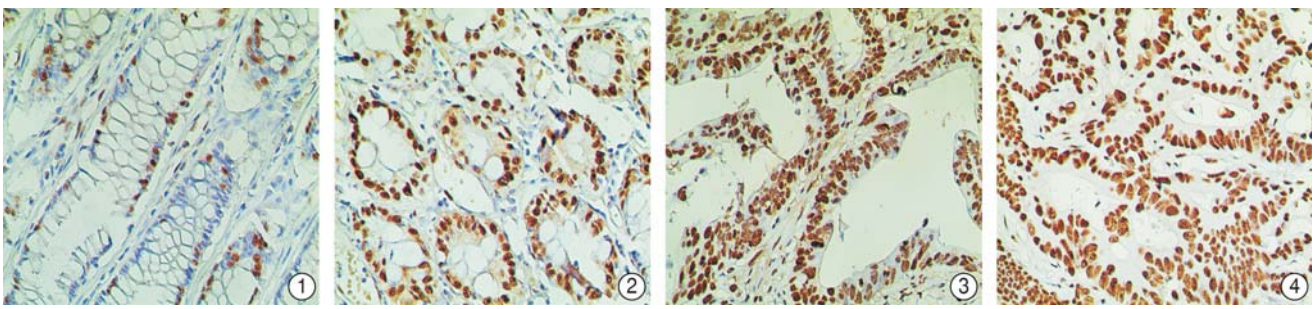

Fig. 1 Nucleolin immunostaining in cancer-adjacent colon mucosa (SP, 400×).
Fig. 2 Nucleolin immunostaining in colon adenoma (SP, 400×).
Fig. 3 Nucleolin immunostaining in well-differentiated colon adenocarcinoma (SP, 400×).
Fig. 4 Nucleolin immunostaining in poorly differentiated colon adenocarcinoma (SP, 400×).

Table 1 Nucleolin immunostaining scores in colon adenocarcinoma and colon adenoma

Groups	*n*	Immunostaining score ($\bar{\chi} \pm s$)
Colon adenocarcinoma	80	7.8 ± 0.1
Colon adenoma	60	6.3 ± 0.2*
Cancer adjacent colon mucosa	50	5.4 ± 0.1*#

Note: Compared with colon adenocarcinoma, * $P < 0.01$; Compared with colon adenoma, # $P < 0.01$

Table 2 Nucleolin immunostaining scores in colon adenocarcinomas with different grades

Groups	*n*	Immunostaining score ($\bar{\chi} \pm s$)
Grades I and II	50	6.8 ± 0.2
Grade III	30	8.0 ± 0.1*

Note: Compared with colon adenocarcinoma Grades I and II, * $P < 0.01$

Discussion

Nucleolin not only functions in a number of fundamental processes, but also in malignant transformation of tumor cells. The overexpression of nucleolin in proliferative and malignant cells indicates that the protein plays an important role in carcinogenesis in some cancers. Astrocytes in the normal human brain do not express nucleolin at a significant level, whereas glioblastoma cell lines and primary human astrocytoma cells exhibit considerable nucleolin expression [3]. Nucleolin expression levels in benign melanocytic lesions are lower than those in dysplastic and malignant melanocytic lesions [8]. A previous study has shown that nucleolin expression levels are significantly higher in atypical and anaplastic meningiomas than in benign meningiomas, and are positively correlated with the Ki-67 labeling index [7]. The total cellular nucleolin level is higher in B-cell chronic lymphocytic leukemia cells than in normal B cells, primarily as a result of the much higher level of cytoplasmic nucleolin in the former cell type [5]. Given its positive correlation with cell growth, the particularly high expression of nucleolin in malignant tumor cells is not surprising. In the present study, the nucleolin immunostaining score in colon adenocarcinoma was significantly higher than those in colon adenoma and cancer-adjacent colon mucosa. The nucleolin immunostaining score in colon adenoma was significantly higher than that in cancer-adjacent colon mucosa. These data indicate that the nucleolin expression level is elevated during the development of colon adenocarcinoma.

The overexpression of nucleolin is not only related to malignancy, but also to a poor clinical prognosis for certain cancer types. In prostate carcinomas, a high nucleolin expression level is correlated with a higher incidence of lymph node metastasis and a higher clinicopathological stage [10]. Cell surface-localized nucleolin expression in prostate cancer cells is elevated and acts as hepatocyte growth factor receptor during cancer progression [11]. The abnormal nucleolin staining patterns in melanoma are correlated with tumor progression and predict a worse prognosis [8]. A reduction in nucleolin expression by siRNA knockdown in a glioblastoma cell line caused a dramatic decrease in cell proliferation, induced cell cycle arrest *in vitro*, and caused a dramatic reduction in tumor size in nude mice [3]. In the current study, further analysis of nucleolin expression with respect to tumor histological grade revealed that the nucleolin immunostaining score in high-grade colon adenocarcinoma was significantly higher than that of low-grade colon adenocarcinoma. These data demonstrated that high nucleolin expression levels in colon adenocarcinoma were correlated with a worse prognosis. Accordingly, we postulate that nucleolin overexpression is an important molecular event during the carcinogenesis of colon adenocarcinoma.

The mechanism by which high nucleolin expression predicts a poor prognosis is presently unclear. It has been documented that anti-nucleolin nonantisense G-rich oligonucleotides could inhibit proliferation and induce apoptosis in cell lines derived from solid tumors and leukemias [4–5]. A previous study has shown that the knockdown of nucleolin in HeLa cells leads to cell cycle arrest and increased apoptosis [12]. Subcellular localization of nucleolin in epithelial cells is Rb-dependent and correlated with nucleolin/DNA binding activity [13]. Nucleolin contributes to the activation of c-myc gene and promotes the induction of B-cell lymphomas [14–15]. Our previous study demonstrated that the overexpression of nucleolin plays an important role during the carcinogenesis of cervical squamous cell carcinoma, and high nucleolin expression levels in cervical intraepithelial neoplasia and cervical squamous cell carcinoma are positively correlated with tumor progression [6]. In addition to nucleolin, CD133 and β-catenin expression levels in tumor cells have significant impacts on prognosis in colon cancer [16].

Taken together, we found that nucleolin expression levels differed significantly between colon adenoma and colon adenocarcinoma, and the nucleolin expression level was positively correlated with the histological grade of colon adenocarcinoma. We postulate that the increased nucleolin expression level plays an important role in the pathogenesis of colon adenocarcinoma and predicts a poor prognosis.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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