Human Apurinic Endonuclease 1 Expression in a Colorectal Adenoma-Carcinoma Sequence

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ABSTRACT

Human apurinic endonuclease 1 (HAP1) plays a key role in the repair of baseless sites in DNA. HAP1 is also known to be a potent regulator of the binding activity of a number of transcription factors. We have examined the immunohistochemical expression of the HAP1 protein in normal colorectal mucosa, hyperplastic polyps, tubulovillous adenomas, and carcinomas. In normal colorectal mucosa, the predominant staining was nuclear in the less differentiated cells located at the lower part of the crypt, but it was cytoplasmic in the more differentiated superficial colonic epithelium. HAP1 expression was nuclear in 3 of 30 adenomas (10%) and 5 of 44 carcinomas (11%), but it was cytoplasmic in 11 of 30 adenomas (37%) and 22 of 44 carcinomas (50%) and both nuclear and cytoplasmic in 16 of 30 adenomas (53%) and 17 of 44 carcinomas (39%). The observed staining in stromal fibroblasts and endothelial cells was nuclear, whereas in macrophages was cytoplasmic. Our data indicate that HAP1 is expressed in different subcellular compartments during normal differentiation and that this pattern is disrupted in adenomas and carcinomas. The differential localization may be relevant to the two different proposed functions of HAP1.

INTRODUCTION

A common insult to cellular DNA is the continuous loss of bases, either spontaneously through hydrolytic depurination and free radical attack or by the action of DNA glycosylases that remove various altered bases (1–3). The resulting AP^3 sites, if left uncorrected, can result in cell death, mutation, and neoplastic transformation. HAP1 [also known as apurinic endonuclease (APE), redox factor 1 (Ref-1), and apurinic excision enzyme (APEX)] is a multifunctional DNA repair enzyme, catalyzing the initial step in AP site repair in human cells by rapidly introducing a hydrolytic cleavage 5' to the site (4–8). The resulting sugar-phosphate residue is subsequently removed by a phosphodiesterase and the one-base gap is filled by a DNA polymerase. The nick is then repaired by the DNA ligase, thus restoring the correct DNA sequence. It has been estimated that up to 10^5 bases are lost per mammalian cell per day by spontaneous depurination (9). This high rate, additionally increased by exposure to certain DNA-damaging agents, is indicative of the significance of an intact DNA repair process in maintaining genetic integrity.

Apart from its role as a DNA repair protein, HAP1 is also known to regulate the DNA-binding activity of several transcription factors, including AP-1, Myb, and nuclear factor KB (8, 10–13). HAP1 enhances the binding of these factors to certain DNA sequences, maintaining the redox status of specific cysteine residues (10, 14). These DNA repair and transcription regulation activities of HAP1 are located in different domains of the enzyme and function separately (14, 15).

Colorectal tumors provide an excellent model for studying the genetic alterations involved in the development of a common human neoplasm. Abundant clinical and histopathological data suggest that most, if not all, malignant colorectal tumors (carcinomas) arise from preexisting benign tumors (adenomas; Refs. 16 and 17). Furthermore, both hereditary and environmental (especially dietary) factors contribute to the development of colorectal neoplasia (18–20). The association of human nonpolyposis colorectal cancer with mutations in human mutS homologue 2 (hMSH2) has been established recently (21–23). It has also been shown that a proportion of patients with early-onset colorectal cancer, but no family history, also carry constitutional mismatch repair gene mutations (24). Thus, defects in DNA repair either predispose to colon cancer or develop during colon cancer progression. Oxygen free radicals and methylating agents generate baseless sites in DNA, and therefore, it is possible that downstream defects in the base excision repair pathway increase the frequency of colon cancer development in those patients. In the present study, using a recently generated antibody to HAP1 and immunohistochemistry, we examined the expression of HAP1 in colorectal adenomas and carcinomas, as well as in normal colonic epithelium and hyperplastic polyps.

PATIENTS AND METHODS

Patients and Tumors. Our material consisted of 44 sporadic colorectal carcinomas (including 36 well- to moderately differentiated and 8 poorly differentiated carcinomas in 31 males and 13 females, ages 48–77 years), 14 hyperplastic polyps, and 20 samples of nonneoplastic colonic mucosa, taken separately at least 5 cm from the edge of the tumor. Thirty of the carcinomas were selected to include both the adenomatous and the carcinomatous components of the tumor on the same tissue section. Nineteen of the adenomas were tubular and 11 were villous, and they measured between 0.8 and 5.7 cm in maximum diameter. The hyperplastic polyps measured less than 0.5 cm. Diagnosis was based on morphological examination of H&E-stained sections. Classification of adenomas was done according to their architecture and degree of dysplasia. Carcinomas were classified according to their degree of differentiation, and for tumor staging, Duke’s classification was followed.

Immunohistochemistry. To determine the cellular expression of HAP1 in tissue material, we used a HAP1-specific polyclonal antibody. Polyclonal anti-HAP1 antiserum (HAP1 antibody 13) was obtained from rabbits after six injections each of 100 μg of recombinant HAP1 protein (14). The antiserum was tested for specificity by Western blotting of whole cell extracts from HeLa cells. Immunohistochemical staining was performed using a standard avidin-biotin-peroxidase complex technique. Before incubation with primary antibody, sections were subjected to two successive microwave irradiations for 5 min each in a heat-stable glass dish filled with 10 mM citrate buffer, pH 6.0, for antigen retrieval. All incubations were performed at room temperature, and Tris-buffered saline, pH 7.2, was used for all washings between incubations. Negative controls consisted of substitution of the primary antibody with an irrelevant antibody or preimmune serum. The specificity of the staining was also confirmed by preincubating the antibody with purified antigen (added in 5-fold molar excess) for 1 h. This resulted in almost complete absorption of the antibody and inhibition of the tissue reaction.

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3 The abbreviations used are: AP, apurinic-apyrimidinic site; HAP1, human apurinic endonuclease 1.
RESULTS

Positive labeling for HAPI was detected in all normal colonic mucosa, all hyperplastic polyps, all adenomas, and all adenocarcinomas. The staining was evident in the nucleus, in the cytoplasm, or in both locations. Details of the staining pattern observed in the tissues examined are described below.

HAPI Expression in Normal Colorectal Mucosa

For most of the 20 cases with normal colonic epithelium, a nuclear pattern of staining was observed for the majority of cells located at the bottom of the crypts (Fig. 1, A and B). As cells differentiated and migrated to the middle zone of the crypt, the nuclear staining gradually decreased. For the more differentiated cells of the surface epithelium, the staining was mainly cytoplasmic (Fig. 1A). Stromal fibroblasts demonstrated a nuclear expression of HAPI, whereas the staining in all the surrounding macrophages was uniformly cytoplasmic. A proportion of the tissue vessels (40%) also showed a weak reaction, which was nuclear for the endothelial cells and cytoplasmic for the vascular smooth muscle cells.

HAPI Expression in Polyps

Hyperplastic Polyps. The expression and localization of HAPI in all 14 hyperplastic polyps examined were similar to those observed in normal colonic mucosa, with the exception of two cases that presented a mixed cytoplasmic and nuclear pattern of staining from the bottom of the crypts to the luminal surface.

Tubular and Villous Adenomas. Adenomas showed all patterns of expression for HAPI. Uniform cytoplasmic staining was detected in 11 of 30 cases (37%; Fig. 1C), and uniform nuclear staining was seen in 3 of 30 cases (10%). In the remaining 16 of 30 cases (53%), the staining for HAPI was both nuclear and cytoplasmic (Fig. 1D).

HAPI Expression in Carcinomas

A similar pattern of expression to that demonstrated for adenomas was obtained for HAPI in colorectal carcinomas. Cytoplasmic staining was seen in 22 of 44 cases (50%), and nuclear staining was seen in 5 of 44 cases (11%; Fig. 1E). Mixed nuclear and cytoplasmic staining was observed in 17 of 44 cases (39%; Fig. 1F). In 27 of the 30 carcinomas (90%) that included both the adenomatous and the carcinomatous components of the tissue in the same section, the staining pattern of HAPI was identical. Fibroblasts, macrophages, and tissue vessels in the stroma of the aforementioned tumors (polyps and carcinomas) showed staining for HAPI similar to that described in normal colonic epithelium.

DISCUSSION

In the present study, we have examined the immunohistochemical expression of HAPI in colorectal adenomas and carcinomas, hyperplastic polyps, and normal colonic mucosa. Our results indicate that HAPI is not expressed uniformly in normal tissues but varies, depending on both the cell types and the stage of cellular differentiation. This may reflect differences in function.

Fig. 1. HAPI streptavidin-biotin immunoperoxidase staining. A, normal colon showing cytoplasmic staining on the luminal surface and nuclear staining in the lower compartment; B, nuclear staining of the cells located at the base of the crypt; C, adenoma demonstrating cytoplasmic staining only; D, adenoma with nuclear and cytoplasmic staining; E, carcinoma with nuclear staining; F, mixed nuclear and cytoplasmic staining in a carcinoma.
In normal colonic mucosa, the nuclear staining observed at the lower part of the crypts may reflect an increased requirement for DNA repair (because this is the replicative compartment of the mucosa), with an increased risk of replicative errors (such as dUTP incorporation), requiring the base excision repair protein. As cells migrated to the upper parts of the crypt and differentiated, the nuclear staining gradually decreased and was replaced by cytoplasmic staining. This cytoplasmic localization of HAP1, in the well-differentiated surface colonic epithelium, possibly indicates a decreased requirement for DNA repair, because these cells have a limited life span.

Similar results were reported for the duodenum by Duguid et al. (25). They showed that there was strong nuclear staining in the crypts and proximal villi but no staining in the distal villi. This shows that there is a marked change during differentiation in both the proximal and distal intestine, which is in contrast to other DNA repair enzymes.

This differential localization was also seen in other normal tissues, in that macrophages uniformly had cytoplasmic staining and fibroblasts had nuclear staining. In tissue vessels, nuclear staining was detected for endothelial cells, and cytoplasmic staining was detected for the vascular smooth muscle cells. HAP1 has a nuclear localization signal, so it is not clear why it can be retained in the cytoplasm in some cell types or tissues. This may reflect further regulation of HAP1 and a cytoplasmic localization related to protection of newly synthesized transcription factors from oxidative damage. The luminal colon epithelium and macrophages, as well as endothelial cells, will be exposed to such challenges. Previous studies have demonstrated an induction of HAP1 in hypoxic conditions (26, 27). Thus, it is possible that high levels of HAP1 are required for the correction of DNA errors caused by the reoxygenation of cells in a hypoxic environment.

AP sites are generated during the repair of methylated bases. There is evidence that colon cells may be particularly exposed to methylating agents. The relationship between dietary saturated fat and high concentrations of fecal bile acids with colorectal cancer is well established (28, 29). Bile acids can undergo N-nitrosoation to form nitrosamides, analogues of N-methyl-N-nitrosourea and N-methyl-N-nitro-N-nitrosoguanidine, which introduce relatively large amounts of methylation damage into DNA (30). Nitrosated bile acid conjugates represent a possible source of endogenous DNA damage to which colon cells may be constantly exposed. Differences in localization of HAP1 may affect the ability of colon cells to repair this type of damage. Because in normal colonic mucosa it is the proliferating compartment that expresses nuclear HAP1, it may be that these cells are better able to protect themselves against the cytotoxic and mutagenic effects of gut toxins.

In hyperplastic polyps, the HAP1 expression phenotype was generally similar to that of the normal luminal epithelium. However, in adenomas and carcinomas, there was a disruption of pattern not related to adenoma/carcinoma progression, and it therefore occurred at an early stage of carcinogenesis. This change in pattern may be due to either the loss of normal cell adhesion or to changes in cellular differentiation pathways that accompany the progression from benign to neoplastic tissue. Controls over the normal expression of HAP1 are lost at the earliest stage of detectable neoplasms (e.g., adenomatous polypsis coli mutations). It will be of interest to analyze the patterns of HAP1 expression in cell lines with such mutations.

Although defects in another DNA repair pathway, mismatch repair, are common in sporadic and familial colon cancers, this did not appear to be the case for AP site repair. The maintenance of HAP1, when downstream mutations (e.g., polymerase-β mutations) in this repair pathway occur, suggest another important role (e.g., maintenance of transcription factor function).

Our study suggests that subcellular localization of HAP1 in normal colonic mucosa during differentiation may be related to different roles of this bifunctional repair protein. This pattern is disrupted at an early stage in the adenoma carcinoma sequence. Although this enzyme was present in all colon tumors, 50% of cases did not have nuclear staining, and whether these will be predisposed to cumulative DNA damage and poor prognosis will be of interest in larger prospective studies.

REFERENCES

HAP1 IN COLON ADENOMA-CARCINOMA


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