in vivo *29*: 391-394 (2015)

Maspin Highlights Colorectal Serrated Polyps: Preliminary Findings

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Abstract. *Aims: Maspin, a 42-kDa serine proteinase inhibitor, is a tumor suppressor protein that stimulates apoptosis and inhibits motility, invasion and cancer metastasis. Mutant maspin leads to partial loss of tumor suppressor function, decreased susceptibility to apoptosis and to malignant progression. We recently found maspin expression (ME) in the cytoplasm of serrated colonic lesions, such as hyperplastic polyps (HP) and sessile serrated adenoma/polyps (SSA/P). Materials and Methods: ME was investigated in 10 colorectal lesions: three HP, three SSA/P and four conventional colorectal adenomas (CCRA). Results: Widespread cytoplasmic ME (comprising the entire height and width of the lesion) was present in the three HP and in the three SSA/P. In contrast, ME was only focally expressed in the four CCRA. ME was not present in the normal mucosa adjacent to those lesions. Conclusion: These preliminary findings suggest that maspin might be of help in discriminating between serrated colonic lesions (HP and SSA/P) and CCRA.*

For many years it was generally accepted that almost all colorectal carcinomas (CRC) evolved from precursor mucosal lesions currently called conventional colorectal adenomas (CCRA) (1, 2). This event is known as the "adenoma-carcinoma pathway". In this model, hyperplastic polyps were considered harmless. More recent studies indicated, however, that the molecular aberrations occurring in serrated colorectal lesions, such as hyperplastic polyps (HP) and sessile serrated adenomas/polyps (SSA/P) (3) are different from those in CCRA.

Maspin, a 42-kDa serine proteinase inhibitor, is a tumor suppressor protein that stimulates apoptosis and inhibits motility, invasion and metastasis (4). When maspin is mutated, it leads to partial loss of tumor suppressor function, decreased susceptibility to apoptosis and malignant progression. Maspin expression has been reported in breast (5), pancreas (6), lung (7) and soft tissue tumors (8).

Payne *et al*. (9) found maspin expression in the luminal cells of hyperplastic/ adenomatous polyps and colonic adenocarcinomas. In contrast with the results of Payne *et al*. (9), we recently found widespread cytoplasmic maspin expression, embracing the entire thickness and length of HP and SSA/P, but not in CCRA.

This study reports these preliminary observations.

Materials and Methods

Sections from 10 colorectal lesions were investigated: three HP, three SSA/P and four CCRA.

HPs were characterized by elongated and funnel-shaped crypts with orderly maturation from crypt base to surface with epithelial serration in the upper and mid-crypt. SSA/P showed distorted crypts, with their basal aspect being dilated or bifurcated, adopting sometimes "L" or "boot" shapes (10). The basal half of the crypts was often serrated, lined with mucinous, mature goblet cells. Excessive extracellular mucin fills the lumen of the dilated crypts and coats the surface of the polyp.

Diagnostic blocks were cut at 4 μm, stained with hematoxylin and eosin (H&E) and immunostained with anti-maspin (H-130 conjugate): sc22762, dilution 1:100 (Santa Cruz Biotechnology, Inc., Carpinteria, CA, USA). The preparations were incubated for 30 min on a Leica Bond MAX instrument (Leica Microsystems, Kista, Sweden). A section without primary antibody (negative control) was included in each preparation.

The topographic distribution of maspin expression (ME) in the cytoplasm was classified into widespread (comprising the entire height and width of the lesion), focal (present in one or more "hot spots") or negative. Only those preparations exhibiting marked ME in the cytoplasm were recorded.

Results

Intense and widespread ME was recorded in all three HP and in all three SSA/P (Figure 1) but not in the four CCRA. In the latter lesions, ME was focally distributed (Figure 1) or negative. In the normal colonic mucosa, adjacent to HP, SSA/P and CCRA, ME was not found.

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Key Words: Maspin, serrated polyps, colon, rectum, conventional adenomas.

Discussion

In a previous publication on ME in the colonic mucosa, Payne *et al*. (9) studied 131 colonic tissues, including hyperplastic/adenomatous polyps and colonic adenocarcinomas. The presentation of the results, however, did not permit for evaluation of the findings (9). Moreover, the extension of ME in those lesions was not given. Nevertheless, judging from their illustrations, focal or diffuse ME in the cytoplasm was only found in the superficial aspect of hyperplastic polyps and tubular adenomas. In another study, Zheng *et al*. (10) found high maspin expression in 41% (n=9) of 22 adenomas adjacent to carcinomas and in 60% (n=71) of 119 adenocarcinomas. The extension of ME was regretfully not given and the captions in the illustrations did not match the interpretation given in the text (10). In the third study on ME in the colonic mucosa, Hestetun *et al*. (11) studied 380 patients with stage II and III colorectal cancer randomized to adjuvant chemotherapy with fluorouracil and levamisole (5-FU/Lev) or to surgery only (control). The authors found that low nuclear ME in colon cancer was an independent predictor of benefit from adjuvant chemotherapy with 5-FU/Lev (11). From the above, it becomes apparent that none of the three previous studies on ME in colonic lesions included SSA/P.

In this preliminary work we found widespread ME in serrated colonic lesions, such as HP and SSA/P. On the other hand, ME was focally distributed or negative in CCRA. The reason(s) for the focal or negative ME in CCRA remains elusive. However, one of the following options should be considered: i) maspin was elaborated but only partially in some areas or ii) maspin was elaborated in very low amounts evading, thereby, the detection by maspin immunostaining. Whichever the molecular mechanism(s) involved, these preliminary results seem to indicate that ME is unpredictable in CCRA. Importantly, the normal colorectal mucosa, adjacent to HP, SSA/P or CCRA, was maspin-negative.

Jang *et al*. (12) found mutated maspin (*SERPINB5*) in 89% of gastric cancers; in human cell lines and in cancer tissues they recorded residue Pro176-maspin frequently mutated to serine, a setting that resulted in decreased apoptosis, enhanced colony formation *in vitro* and increased tumorigenesis *in vivo*. In a mouse model, cells expressing Ser 176-maspin showed a higher rate of tumor formation *in vivo* than those expressing Pro176-maspin. Jang *et al*. (12) postulated that P176S (C526T) substitution of maspin might lead to a partial loss of the tumor suppressor function of the protein, thus contributing to decreased susceptibility to apoptosis and to malignant progression. Against this background, we are inclined to speculate that the widespread (and intense) ME recorded in colorectal HP and SSA/P might be a manifestation of mutated maspin. Further studies are necessary to confirm whether maspin immunostaining is a suitable candidate marker for monitoring the "serrated pathway" of colorectal carcinogenesis.

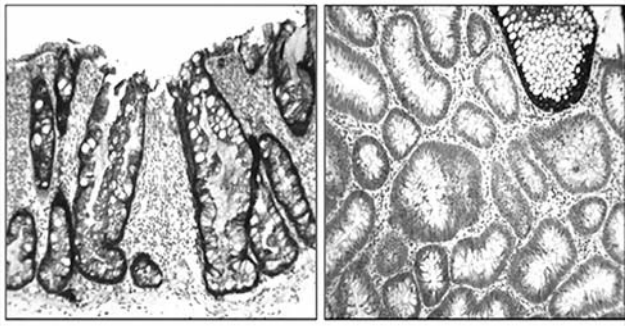

Figure 1. *Left panel: Sessile serrated adenoma/polyp (SSA/P) showing widespread maspin expression. Right panel: Conventional colonic adenoma showing focal distribution of maspin-expressing adenomatous tissue (magnification, ×10).*

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Received January 23, 2015
Revised February 16, 2015
Accepted February 17, 2015