Maspin, a Marker of Serrated Colorectal Polyps

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Abstract. *The serine proteinase inhibitor maspin is a tumor-suppressor protein that stimulates apoptosis and inhibits motility, invasion and cancer metastasis. Mutant maspin galvanises partial loss of tumor-suppressor function, reducing susceptibility to apoptosis and facilitating malignant progression. Mutant maspin has been reported in many tumor types. We recently analyzed maspin expression in 128 colorectal lesions: 39 hyperplastic polyps (HPs), 29 sessile serrated adenoma/polyps (SSA/Ps), three traditional serrated adenomas (TSAs), 20 conventional colorectal adenomas (CCRAs), 5 carcinomas evolving from CCRA, 12 active inflammatory bowel disease (IBD), 2 ulcerative colitis (UC) in remission, 4 solitary ulcers (rectum) and 12 normal colorectal mucosa. The topographic distribution of maspin in the cytoplasm was classified into i) extensive, ii) focal, or iii) negative. The intensity of maspin expression in the cytoplasm was classified into i) unquestionable or ii) negative. Cases with faint (questionable) maspin expression were also recorded as negative. Extensive maspin expression was recorded in 95% (39/41) of the HPs, in 100% (29/29) of the SSA/Ps (including one carcinoma arising in a SSA/P), in 66% (2/3) of the TSAs, but only in 10% (2/20) of the CCRAs. None of the specimens with carcinoma arising in CCRA, with UC in remission or with solitary ulcer exhibited extensive maspin expression. Importantly, maspin was not expressed in the normal mucosa (including that adjacent to HP, SSA/P, TSA and CCRA). It is submitted that extensive maspin expression might be a manifestation of mutant maspin in lesions central to the serrated pathway of colorectal carcinogenesis.*

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Colorectal carcinoma, the third most commonly diagnosed type of cancer in Europe and in the USA (1) usually evolves *via* the adenomatous polyposis coli (APC) pathway along the conventional adenoma–carcinoma sequence. Conventional colorectal adenomas exhibit tubular or villous structures either with low- or high-grade dysplasia (2, 3), progressive accumulation of *KRAS* mutations and chromosomal instability (3). In this paradigm, hyperplastic polyps are considered innocuous.

Most recently, the serrated pathway of colorectal carcinogenesis has been widely accepted (4, 5). In this model, hyperplastic polyps (HPs), sessile serrated adenoma/polyps (SSA/Ps) and traditional serrated adenomas (TSAs) are regarded as early histological precursors of serrated carcinomas. The main molecular aberrations found in SSA/Ps are *BRAF* mutations and DNA methylation, leading to the CpG island methylator phenotype, microsatellite instability and loss of DNA mismatch-repair proteins, particularly MutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*), known as MLH1, and postmeiotic segregation increased 2 (*S. cerevisiae*), known as PMS2 (6-8). It has been claimed that progression to colonic cancer through this pathway occurs at a faster rate than *via* the conventional adenoma–carcinoma pathway (7). The serrated pathway accounts for approximately 15-30% of all colorectal carcinomas (9-11).

Maspin, a 42-kDa serine proteinase inhibitor, is a tumor-suppressor protein that stimulates apoptosis and inhibits motility, invasion and cancer metastasis. It was first identified in human breast carcinoma (12) and subsequently in pancreatic (13) and lung (14) cancer, in soft tissue tumors (15), and in colorectal adenomas and carcinomas (16, 17). More recently, Payne *et al*. found maspin expression in the luminal cells of HP/adenomatous polyps, in high-grade tumors and in the colonic tissue from a patient with ulcerative colitis having adenocarcinoma (18).

While studying sub-epithelial myofibroblasts in serrated colorectal polyps (19) with the aid of maspin, α-smooth muscle actin, vimentin, and cyclo-oxygenase-1 and -2 enzymes, we noticed that the cytoplasm of serrated colorectal polyps expressed maspin while the normal mucosa remained unstained (20). For the present study, consecutive

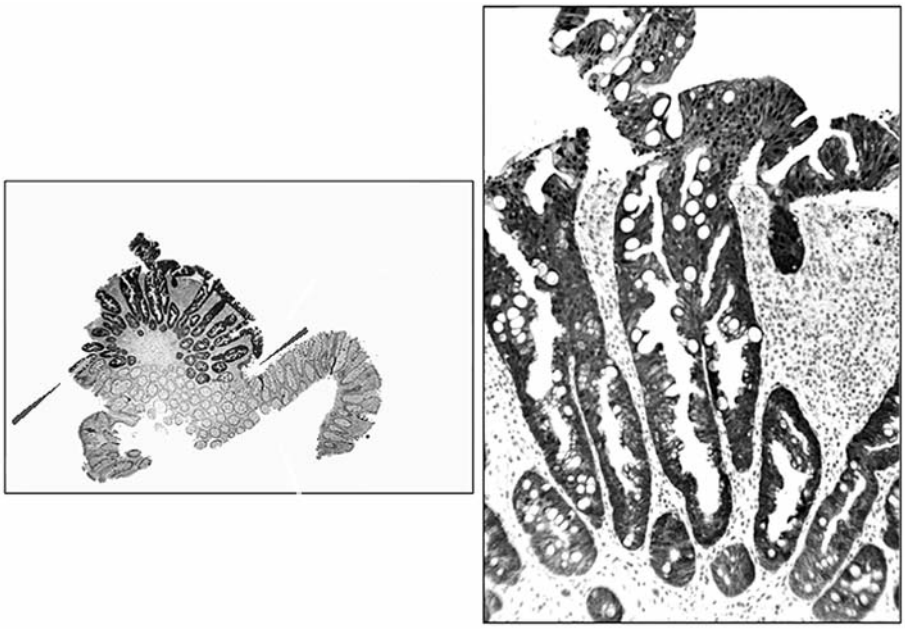

Figure 1. *Left panel: Hyperplastic colonic polyp, showing extensive maspin expression (between arrowheads); note unstained adjacent normal mucosa (×4). Right panel: Detail of the hyperplastic colonic polyp (×10). Maspin immunostain.*

colorectal biopsies diagnosed with colorectal HP, SSA/P and TSA filed at this Department were immunostained for maspin. The results were compared against those found in conventional colorectal adenomas, in colorectal carcinomas, and in normal colorectal mucosa.

Materials and Methods

Sections from 193 colorectal biopsies were investigated: 39 HPI, 29 SSA/Ps, three TSAs, 20 conventional colorectal adenomas, five adenocarcinomas evolving from conventional adenomas, 12 with active inflammatory bowel disease (IBD), two with ulcerative colitis in remission, four solitary ulcers (rectum), and 12 with normal colorectal mucosa (normal donors). In addition, the normal colorectal mucosa adjacent to 65 lesions (25 HPs, 22 SSA/Ps, one TSA, and 17 conventional adenomas) was investigated.

Following the WHO guidelines (21), lesions were classified into HP, SSA/P, TSA.

Immunohistochemistry. Diagnostic blocks were cut at 4 μm, stained with hematoxylin and eosin 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 in the cytoplasm was classified into: i) extensive (*i.e.* present in the entire thickness and length of the lesion), ii) focal (*i.e.* present in one or more 'hot spots'), and iii) negative.

Table I. *Results of 193 colorectal biopsies immunostained with anti-maspin, showing extensive maspin expression (n=76), focal maspin expression (n=31), or no maspin expression (n=86).*

	Extensive maspin expression	Focal maspin expression	No maspin expression	Total
HP	39	2	0	41
SSA/Ps	29	0	0	29
TSA	2	1	0	3
CCRA	2	18	0	20
Cancer in CCRA	0	2	3	5
Active IBD	4	8	0	12
UC in remission	0	0	2	2
Solitary ulcer	0	0	4	4
Normal mucosa	0	0	12	12
Normal mucosa adjacent to*	0	0	65	65
ALL	76	31	86	193

HP: Hyperplastc polyps, SSA/Ps: sessile serrated adenomas/polyps, TSA: traditional serrated adenomas, CCRA: conventional colorectal adenomas, IBD: Inflammatory bowel disease, UC: ulcerative colitis, *25 HP, 22 SSA/P, one TSA, and 17 conventional adenomas.

The intensity of expression was semi-quantitatively classified into: i) unquestionable, and ii) negative. Cases with faint (questionable) expression were also recorded as negative.

Statistical analysis. The non-parametric Mann–Whitney test was used to assess difference between groups. Statistical significance was set at a value of $p<0.05$).

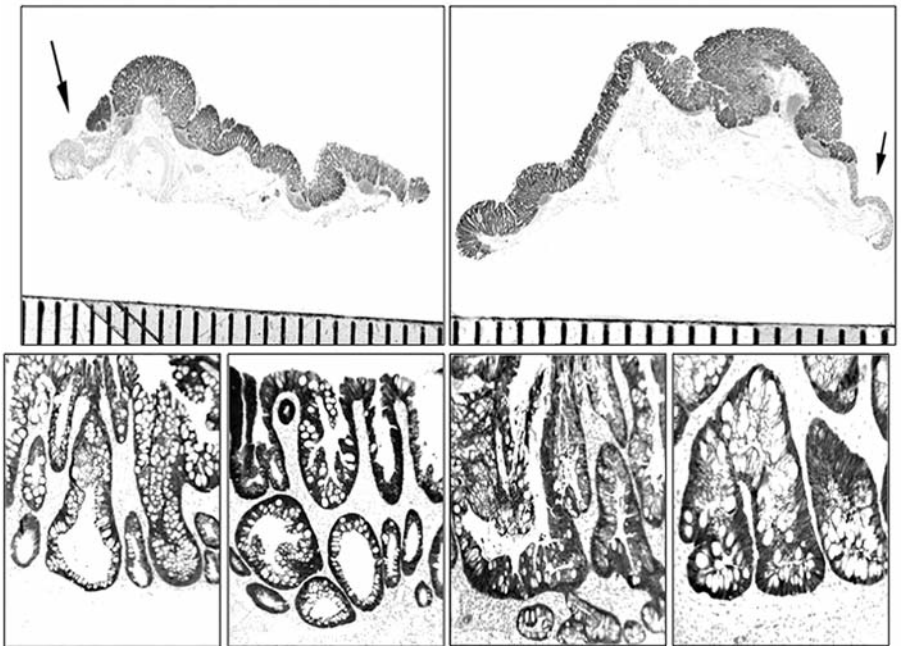

Figure 2. *Sessile serrated, adenomas/polyps (SSA/Ps). Upper panel: Low-power view of two SSA/Ps showing extensive maspin expression; note adjacent maspin-negative normal mucosa (at arrows). Section scanned on an Epson Perfection 4990; the mm scale discloses the actual size of the lesion. Lower panel: Detail showing distorted crypts at the base of SSA/Ps with extensive maspin expression (maspin immunostain, ×20).*

Results

Extensive maspin expression. Results in Table I show that extensive maspin expression was recorded in 95% (39/41) of the HPs (Figure 1), in 100% (29/29) of the SSA/Ps (Figure 2), in two out of three of the TSAs (Figure 3), but only in 10% (2/20) of the CCRAs. In addition, 33% (4/12) of the biopsies with active IBD exhibited extensive maspin expression. In contrast, maspin was not expressed in invasive carcinomas arising from CCRA (0/5), in specimens with UC in remission (n=2) or solitary ulcer (0/4). The difference between extensive maspin expression in HP/SSA-P/TSA *vs*. CCRA/invasive carcinomas arising from CCRA/ulcerative colitis in remission/solitary ulcers was significant ($p<0.05$).

Focal maspin expression. Table I also shows that focal maspin expression was recorded in 5% (2/41) of the HPs, in one out of three of the TSAs, in 90% (18/20) of the CCRAs (Figure 4a), in two out of five of the invasive carcinomas evolving from CCRA and in 67% (8/12) of the cases with active IBD. None of the 29 SSA/Ps, ulcerative colitis in remission (0/2), or solitary ulcer (0/4), exhibited focal maspin expression.

Negative maspin expression. Negative maspin expression was found in three out of the five carcinomas evolving from CCRA (Figure 4b), and in all specimens with ulcerative colitis in remission (2/2), or with solitary ulcer (4/4). Importantly, maspin was not expressed in normal colorectal mucosa, including the normal mucosa adjacent to HP, SSA/P, TSA, and CCRA (72/72) (Table I).

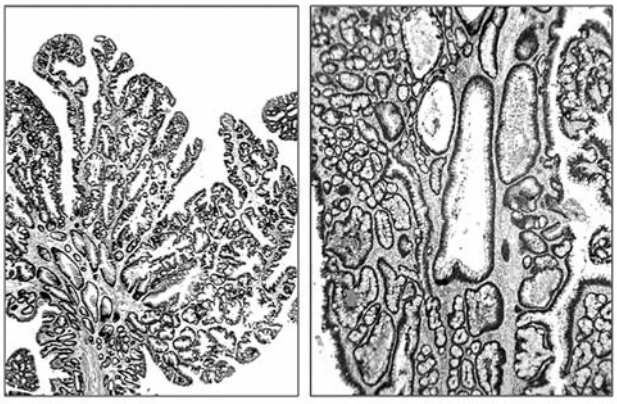

Figure 3. *Left panel: Traditional serrated adenoma showing extensive maspin expression (maspin immunostain, ×4). Right panel: Detail to demonstrate extensive maspin expression in the microtubules or ectopic crypt formations (maspin immunostain, ×10).*

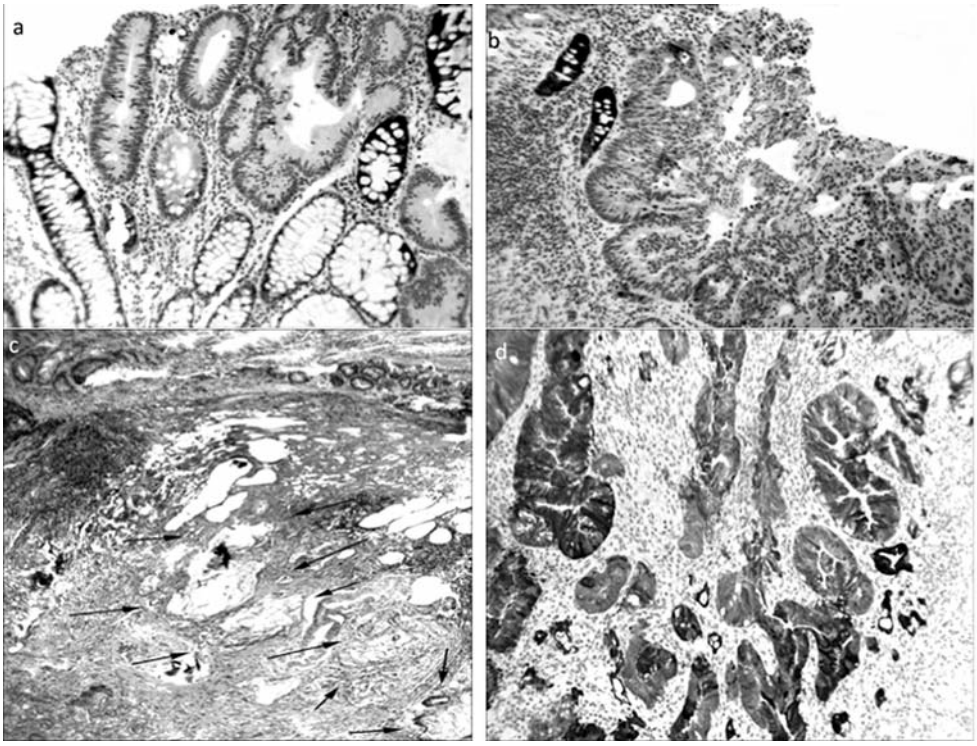

Figure 4. *a: Conventional colonic adenoma showing focally-distributed maspin expression alternating with absence of maspin expression in adenomatous tissue. Note that some apparently non-dysplastic colonic glands within the adenomatous tissue express an enhanced level of maspin (maspin immunostain, ×10). b: Carcinoma evolving from a conventional colonic adenoma. Note absence of maspin expression, except for two apparently non-neoplastic glands (maspin immunostain, ×4). c and d: Adenocarcinoma evolving from a sessile serrated, adenomas/polyp (SSA/P). c: Note mucus-rich adenocarcinoma in the submucosa (arrows, hematoxylin and eosin stain, ×2). d: Extensive maspin expression in an adenocarcinoma evolving from a SSA/P (maspin immunostain, ×20).*

Discussion

The results of the present study indicate that the majority of the colorectal serrated lesions (HP, SSA/P and TSA) displayed extensive maspin expression. In CCRA, maspin was often focally distributed. Although the reason for the latter setting remains elusive, possible alternative explanations might be: i) that maspin was not expressed in certain neoplastic areas, or ii) that it was expensed but in very low amounts, thereby evading detection by the immunostain.

In CCRA and in adenocarcinomas arising in CCRA, occasional crypts with no apparent neoplastic histological changes expressed maspin (Figure 4a and b). The cause of this paradoxical phenomenon remains poorly understood.

The only invasive carcinoma evolving from SSA/P in this series exhibited extensive maspin expression (Figure 4d). Whether this phenomenon is fortuitous or is a common phenomenon present in invasive carcinomas evolving from SSA/P is difficult to assess, considering that only 64 cases of invasive carcinomas evolving from SSA/P have been published in the literature (22-36).

Paradoxically, extensive maspin expression was found in 33% of the biopsies with active IBD. These results, however, are in concordance with those of Cao *et al*. (37); the authors found maspin overexpression in both active IBD and in colitis-associated dysplasia, suggesting a potential role of maspin in neoplastic progression in patients with IBD (37).

Jang *et al*. found mutated maspin in 89% of gastric carcinomas (22). In human cell lines and cancer tissues, they found residue Pro176-maspin frequently mutated to Ser; the mutation resulted in decreased apoptosis, enhanced colony formation *in vitro* and increased tumorigenesis *in vivo*. In a mouse model, cells expressing Ser176-maspin had a higher rate of tumor formation *in vivo* than those expressing Pro176-maspin. Jang *et al*. postulated that the P176S (C526T) substitution of maspin might lead to a partial loss of the tumor-suppressor function of the protein, contributing to decreased susceptibility to apoptosis and to malignant progression (22).

Based on these considerations and bearing in mind that maspin was not expressed in normal colorectal mucosa, we speculate that the extensive maspin expression found in HP,

SSA/P and TSA-ECF might reflect the mutant form of maspin protein.

In conclusion, it is apparent that maspin is an auspicious marker to highlight lesions central to the serrated pathway of colorectal carcinogenesis. The review of the literature indicates that this appears to be the first report on the expression of maspin in SSA/P and TSA.

Conflicts of Interest

None.

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