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INCREASED EXPRESSION OF FAT10 IN COLON BENIGN, PREMALIGNANT AND MALIGNANT EPITHELIAL NEOPLASMS

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Abstract

Over expression of FAT10 is characteristic of numerous types of carcinoma including liver, gastric and colon carcinomas. In the case of colon carcinoma it is possible to determine at the point in the progression from the benign to the malignant process of colon cancer development by determining which stage in the neoplastic process FAT10 overexpression occurs. This stage was determined by measuring the intensity of fluorescence of immunohistochemically stained normal mucosa, tubular adenomas, hyperplastic polyps, serrated adenomas, villotubular, villous adenomas and invasive adenocarcinoma stages. Using this approach it was found the overexpression of FAT10 began at the serrated adenoma stage and continued to include the villous and villotubular stages and the invasive adenocarcinoma stage. The FAT10 overexpression by invasive adenocarcinoma was accompanied by the expression of the catalytic subunits of the immunoproteasome which is functionally tied to overexpression of FAT10, Toll-like receptor activation and the proinflammatory response.

Keywords

FAT10; immunoproteasome; Toll-like receptors; interferon; proinflammatory response

INTRODUCTION

Papers, beginning with Lee et al (2003), have reported that FAT10 is up-regulated in colon cancer (Lukasiak et al, 2008). They reported that FAT10 was overexpressed in 80% of colon cancers. Lukasiak et al, 2008, found that 8 of 15 colon cancers (53%) overexpressed FAT10 as indicated by RT-PCR. The highest increase was 15 fold.

The overexpression of FAT10 by colon epithelial neoplasms suggests that proinflammatory cytokines are involved in the pathogenesis of colon cancer (Lukasiak et al., 2008). This is based on the fact that the FAT10 phenotype is a response to Toll-like receptor ligands and the cytokines IFN- γ and TNF α in liver cancer (Raasi et al., 1999; Bardag-Gorce et al., 2010a; Oliva et al., 2010; Bardag-Gorce et al., 2010b). Both IFN- γ and TNF α induce FAT10 (UBD) together with the immunoproteasome including all three catalytic subunits LMP2-7 and MECL-1 (Lukasiak et al, 2008; Bardag-Gorce et al., 2010a). In the liver FAT10 overexpression is an epigenetic marker for liver neoplasia (Oliva et al 2008; French et al.,

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2010a and b). FAT10 overexpression is associated with a growth advantage of the overexpressing hepatocytes (Oliva et al, 2008). Overexpression of FAT10 correlated with tumor stage and metastasis in gastric carcinoma cases (Ji et al., 2009).

In view of the overexpression of FAT10 as a sign of the influence of proinflammatory cytokines in powering the neoplastic process in the colon, we have systematically immunostained colon neoplasms to determine the level of expression of FAT10 at various stages in the carcinogenic process.

METHODS AND MATERIALS

Random samples of colon biopsy of colon neoplasms at various stages of developments as follows: normal colonic mucosa, hyperplastic polyps, tubular adenomas, serrated adenomas, villotubular adenomas, low grade dysplasias, high grade dysplasias, intramucosal adenocarcinomas, invasive adenocarcinomas were immunostained for FAT10.

Immunofluorescence staining and photography.

The biopsies were stained with a rabbit polyclonal antibody to FAT10 (ENZO Life Science Int Inc, Plymouth Meeting, PA). The second antibody used was donkey anti-rabbit (Jackson Immuno Research Lab, Inc., West Grove, PA) which was labeled with fluorescence isocyanate (FITC). 4',6-Diamidino-2-phenylindole (DAPI) was used as a nuclear stain. The intensity of FITC/FAT10 staining was measured semiquantitatively using 2x objective magnification and a standard brightness setting of 250 ms using a Nikon 400 fluorescent microscope, and the Nikon morphometric system. A colon carcinoma was also stained with rabbit antibodies to the catalytic subunits of the immunoproteasome; LMP2 (Santa Cruz, Santa Cruz, CA), LMP7 (Abcam, Cambridge, MA), and MECL-1 (Santa Cruz, Santa Cruz, CA).

STATISTICAL ANALYSIS

Results are expressed as group Mean \pm S.E.M. Data were evaluated using one-way ANOVA followed by Student's t-test for comparisons between lesional groups and normal controls. Means were considered significantly different at $P < 0.05$.

RESULTS

Role of FAT expression in colonic neoplasms

The role of FAT10 expression of eighteen cases of normal colon mucosa with or without an associated neoplasm, were measured for staining intensity using an antibody against FAT10. The intensity of staining was not significantly different whether there was an associated neoplasm or not (145 ± 6.4 fluorescent intensity units). An example is shown in Fig 1A. The intensity measurements and photographs were always taken using the same brightness setting and the same magnification so as to compare the intensity of the fluorescence of the different colonic neoplastic lesions.

Eight cases of tubular adenoma were measured and the staining intensity for FAT10 was not significantly different when compared with normal mucosa (Fig 1B, Fig 2, 157 ± 4.9 fluorescent units, $P = 0.262$).

Five hyperplastic polyps were measured and the staining intensity for FAT10 was not significantly different when compared with normal mucosa (Fig 1C, Fig 2, 168 ± 9.0 fluorescent units, $P = 0.098$).

Five serrated adenomas were measured and the staining intensity for FAT10 was significantly increased compared to normal mucosa (Fig 1D, Fig 2, 208 ± 8.5 fluorescent units, $P=0.0001$).

Twelve villotubular and villous adenomas (Fig 2E&F) were measured and the staining intensity for FAT10 was significantly increased compared to normal mucosa (Fig 2, 167 ± 5.5 fluorescent units; $P=0.0221$).

Six invasive carcinomas were measured and the staining intensity for FAT10 was significantly increased compared to normal mucosa (Fig 3A, Fig 2, 196 ± 14.1 fluorescent intensity, $P=0.0012$).

Foci of low and high grade dysplasia and intramucosa adenocarcinoma did not appear to differ in fluorescent intensity (Fig 3B) but this was not measured because of the difficulty of locating these foci in the villotubular adenomas when the fluorescent stained specimens were examined.

Role of immunoproteasome catalytic subunits

Immunostains for the immunoproteasome catalytic subunits LMP2 and 7 and MECL-1 were performed on an invasive adenocarcinoma of the colon because the expression of the immunoproteasome subunits are coexpressed with FAT10 in response to the proinflammatory cytokines interferon gamma and TNF α (Lukasiak et al., 2008; Bardag-Gorce et al., 2010). The colon carcinomas cells stained strongly positive with antibodies to LMP2 (Fig 3C), LMP7 (Fig 3D) and MECL-1 (Fig 3E). The glands in the normal colon mucosa were also stained positive (Fig 3F).

DISCUSSION

Fan et al., (2008), using laser capture microdissection and cDNA microarrays, documented overexpression of FAT10 by colon carcinoma cells compared to normal colon mucosa cells. In the present study, using semiquantitative immunohistochemistry for FAT10, we observed a significant increase in staining intensity for FAT10 in serrated adenomas, villotubular adenomas and invasive colon carcinomas. Although tubular adenomas and hyperplastic polyps tended to show an increase in staining intensity compared to normal mucosal cells, the increase was not at a significant level. These results suggest that FAT10 expression is involved in the mechanism of progression from the benign polyps to the development of invasive carcinoma.

FAT10, like the immunoproteasome catalytic subunits, LMP2 and 7 is expressed by a gene located at the major histocompatibility complex class 1 locus on chromosome 6.

(Fan et al., 1996; Bates et al., 1997; Ebstein et al., 2009). The expression of these genes is up regulated by toll-like receptor ligands and the cytokines interferon gamma (IFN γ) and TNF (Oliva et al., 2010; Lukasiak et al., 2008; Raasie et al., 1999; Bardag-Gorce et al., 2010a; Bardag-Gorce et al., 2010b). The expressions of FAT10 is down-regulated by wild type p53 (Zhang et al., 2006) and up-regulated by mutant p53 (Ji et al., 2009). The up-regulation of IFN γ receptors IFN γ R1 and 2 (Bardag-Gorce et al., 2010b) in response to the activation of the toll-like receptors by bacterial, viral or chemical agents in the colon could activate the FAT10 associated up regulation of the immunoproteasome expression (i.e. the catalytic subunits LMP2 and 7 and MECL-1) and the proinflammatory response which leads to the neoplastic transformation of colon epithelium analogous to the same phenomenon in liver carcinogenesis (French et al., 2010b). To test this hypothesis we immunostained a colon carcinoma with antibodies to LMP2 and 7 and MECL-1. The invasive colon carcinoma cells

stained intensity positive for all 3 catalytic subunits suggesting that proinflammatory cytokines are involved in the mechanisms of colon carcinogenesis.

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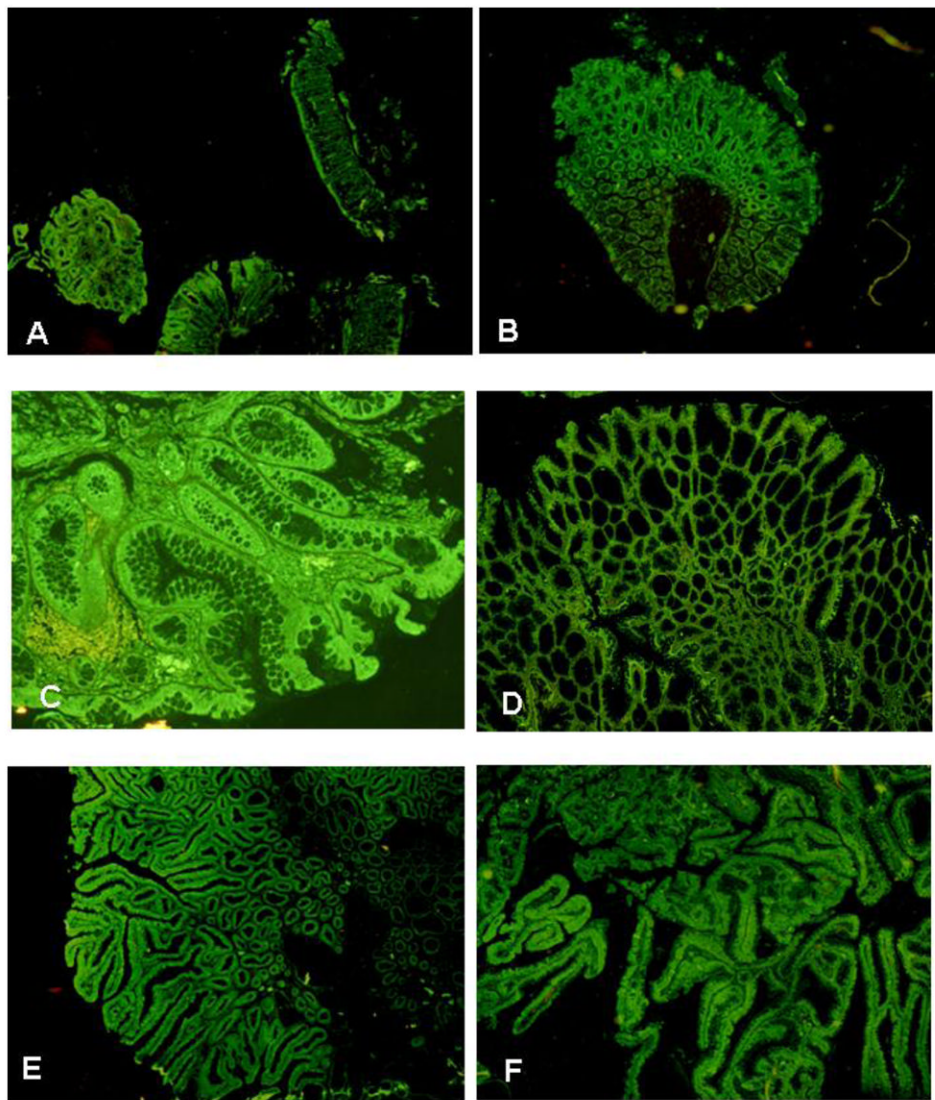


Fig 1.

A. Normal colon mucosa cut at different planes immunostained for FAT10. x22. B. Tubular adenoma immunostained for FAT10. Note that the tubular adenoma at the top stains greener than the normal mucosa located at the bottom of the photo. x22. C. Hyperplastic polyp immunostained for FAT10. x22. D. Serrated adenoma immunostained for FAT10. x22. E. Villotubular adenoma immunostained for FAT10. x22. F. Villous adenoma immunostained for FAT10. x22.

Immunofluorescent Intensity for Fat10

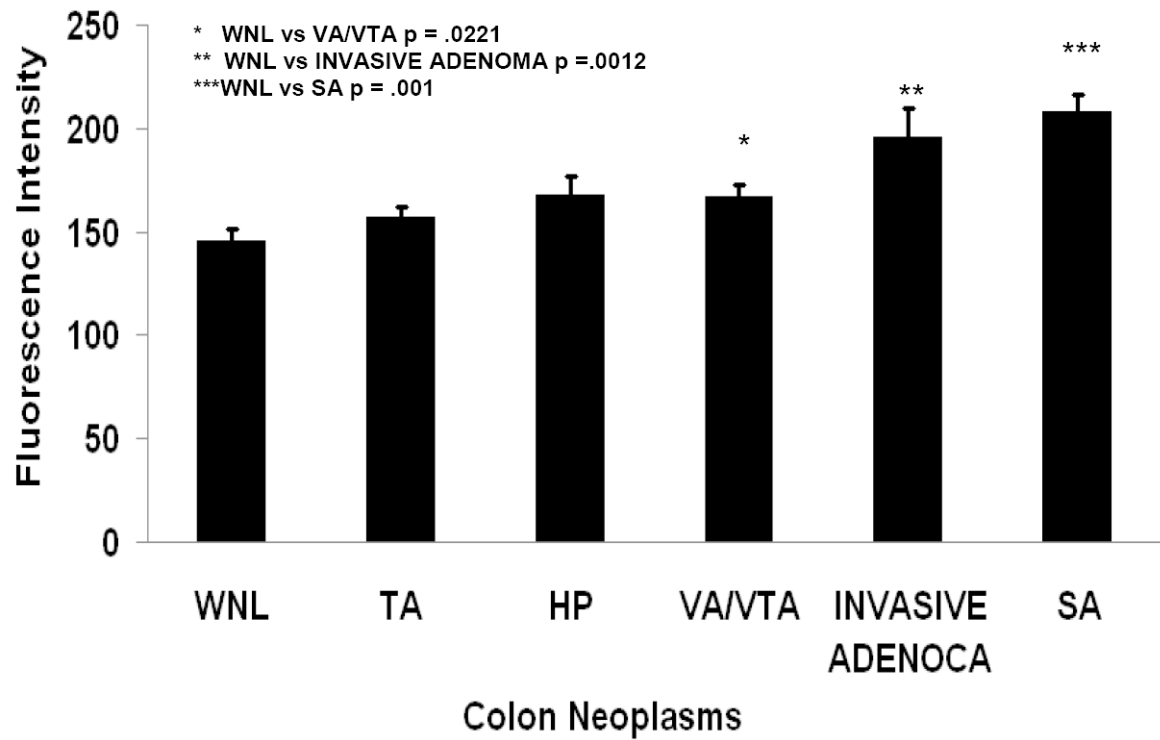


Fig 2. Fluorescent intensity bar graph of the colonic normal mucosa (WNL) and neoplasms. TA (tubular adenoma), HP (hyperplastic polyps), VA/VTA (villous adenoma/villotubular adenoma), SA (serrated adenoma). (Mean ± S.E.M.).

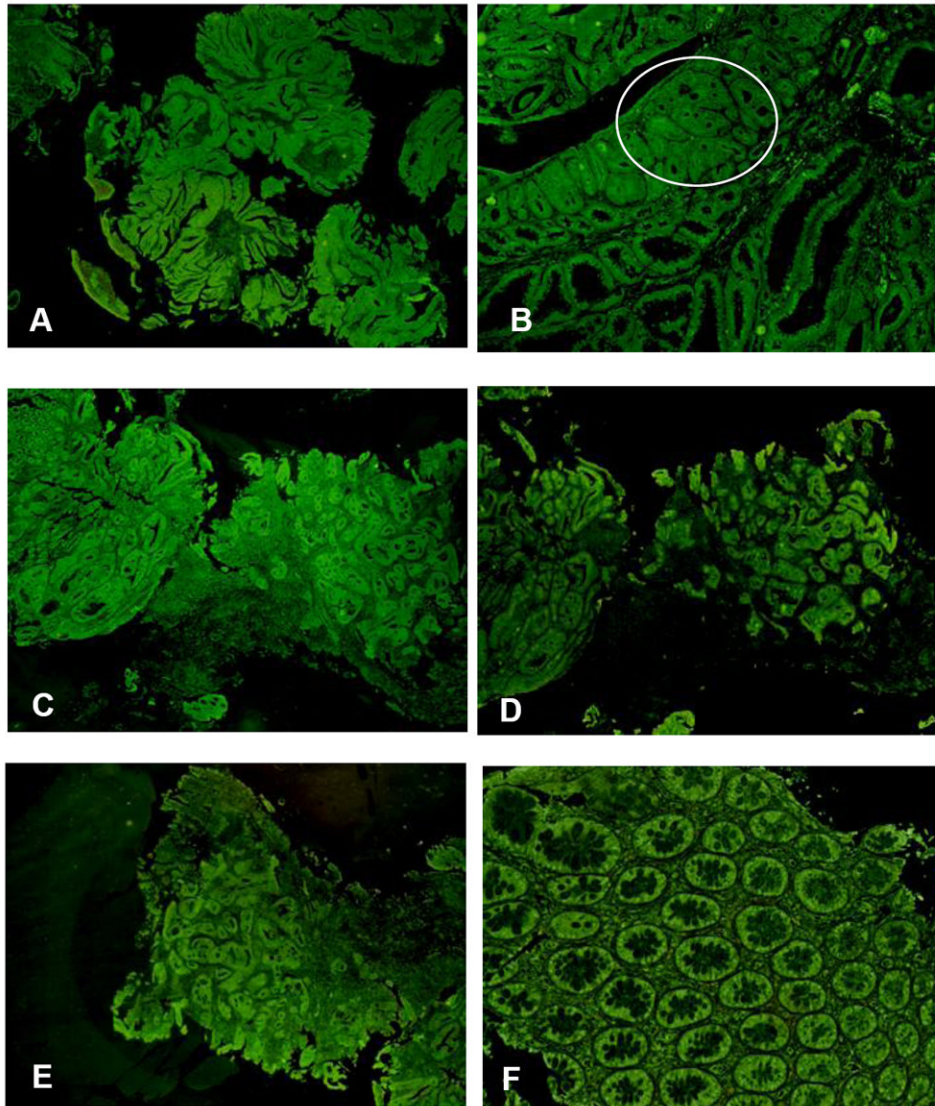


Fig 3.
 A. Invasive adenocarcinoma immunostained for FAT10. x22. B. High grade dysplasia (circle) in a villotubular adenoma. x44. C. Invasive adenocarcinoma immunostained for LMP2. x22. D. Invasive adenocarcinoma immunostained for LMP7. x22. E. Invasive adenocarcinoma immunostained for MECL-1. x22. F. Normal mucosa immunostained for MECL-1. x109.