Can arginase be a marker of the large bowel neoplasia?

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In extrahepatic mammalian tissues in which the urea cycle is not functioning, arginase (EC 3.5.3.1) is believed to supply the cell with ornithine. This non-protein amino acid is a precursor for biosynthesis of the polyamines playing an important role in regulation of cell proliferation and differentiation [1-3].

Cancer results from genetic alterations leading to the progressive disorders of the normal mechanisms controlling growth [4] including regulation of biosynthesis of polyamines which are critical for growth of both normal and transformed cells [5, 6]. It has been shown recently that, in colorectal cancer, the mucosal content of polyamines is markedly elevated as well as the activity of ornithine decarboxylase (EC 4.1.1.17; ODC¹), the key enzyme controlling polyamine synthesis [7-9]. Thus, it seems that the increased level of ornithine due to the elevated arginase activity might be prerequisite for tumor development.

Since most colorectal carcinomas appear to arise from adenomas [8, 10] and tumors at various stages of development can be obtained for analysis, we have attempted to follow changes in arginase activity during tumor progression. Since the relation between colorectal cancer and ulcerative colitis (UC) has now been generally accepted [11], it would be of great clinical and diagnostic value to find any early marker of colorectal cancer in long-term UC. Therefore, our studies were extended to UC-patients at various stages of the disease.

In the presented work the following groups of patients were included: 28 patients with colorectal dysplastic polyps (average age 58 years), 14 patients with colorectal carcinomas (average age 63 years), and a control group of 14 adults (average age 55 years); 28 children with ulcerative colitis (average age 13 years), 20 children with non-specific colitis (average age 9 years) and a control group of 12 children (average age 8 years).

Adenomas (polyps) were removed by endoscopic resection. Specimens of carcinoma tissue were removed endoscopically (n = 6) or surgically (n = 8). The former tumors were histologically graded as slight or moderate dysplasia, and the latter were classified as severe dysplasia. Simultaneously, specimens of the corresponding normal-appearing mucosa were taken from each patient at the distance of about 5 cm from the tumor. Biopsies from intestinal mucosa of children with ulcerative colitis and, for comparison, nonspecific colitis were taken during routine colono- or rectoscopic examinations. According to the results of the endoscopic and histopathological examinations, UC was classified as being in the acute (n = 12), chronic (n = 6) and remission (n = 10) stages. The controls, both in the adult and the children group, were rectal biopsy specimens obtained

¹Abbreviations: ODC, ornithine decarboxylase; UC, ulcerative colitis
at colonoscopic examinations performed for evaluation of occult blood in stools and in each instance the examination results were normal.

All tissue specimens were immediately chilled after removal and assayed within a few hours. Each tissue specimen was weighed and then homogenized in 5 vol. of cold 5 mM Tris/HCl buffer, pH 7.5, containing 1 mM MnCl₂, in a Potter-type homogenizer. Arginase activity was determined in tissue homogenates by the microtechnique described previously [12, 13] and was expressed in units per 1 g of wet tissue or per 1 mg of protein. One unit (U) of arginase activity was defined as one μmol of the liberated ornithine per minute at 37°C. The protein content was measured according to Lowry et al. [14].

The mean activity of mucosal arginase in normal subjects was very low and the difference between adults (1.25 ± 0.51 U/g tissue or 6.5 ± 2.7 mU/mg protein) and children (1.20 ± 0.43 U/g tissue or 6.2 ± 2.2 mU/mg protein) were negligible.

In polyps (adenomas) the arginase activity was significantly elevated as compared with the enzyme activity in the corresponding normal-appearing mucosa of the same subject (Fig. 1).

Carcinoma tissue showed a remarkably high level of the enzyme activity compared with adjacent normal-appearing mucosa (Fig. 1). In the examined carcinomas the activity was at least 10 times as high as in normal mucosa of controls and at least 3 times as high as in adenomas showing a slight degree of dysplasia.

The relation has been proved between the degree of dysplasia and the increase of arginase activity in adenomas and carcinomas. In slight, moderate and severe dysplasia the mean arginase values showed on average an about 2-fold, 5-fold and 20-fold increase, respectively as compared with normal mucosa of controls (Fig. 1). A similar significant increase was also found in the specific activities of arginase. It is noteworthy that in the mucosa adjacent to the investigated lesions, although morphologically normal, arginase activity was significantly higher than in normal mucosa of controls and this elevation was also related to the degree of dysplasia found in the tumor (Fig. 1).

We have found that ulcerative colitis is also characterized by increased activity of mucosal arginase. As shown in Fig. 2 there is an evident correlation between the enzyme activity and the degree of the inflammatory process, assessed by endoscopic and histopathological methods. In acute, chronic and remission stages of UC the mean activities were about 6 times, 4 times and twice as high as in normal mucosa of controls, respectively. In acute UC, the enzyme activity was on average about 3 times as high as in the acute nonspecific colitis.

![Graph showing arginase activity in different conditions](image)

**Fig. 1.** The activity of arginase in colorectal adenomas and carcinomas, and in the adjacent normal-appearing mucosa about 5 cm from the tumor, in relation to the degree of dysplasia found in the lesion.

[ ], Normal-appearing mucosa; [ ], tumor tissue. Means ± S.D. are given.
Fig. 2. The activity of mucosal arginase at different stages of ulcerative colitis in comparison with the enzyme activity in nonspecific colitis and in normal mucosa of controls. Means ± S.D. are given

It should be emphasized that, even in the cases of endoscopic and histopathological remission of the disease, the elevated arginase might be responsible for the increased production of ornithine, an inducer of ODC [15], and the enhancement of polyamine synthesis. From the above results, and those illustrating very high activities of both arginase (Fig. 1) and ODC [9] in colorectal carcinomas, it can be speculated that high incidence of cancer in long-term UC may be related to highly active polyamine synthesis leading to abnormal proliferative status of the intestinal mucosa.

The results presented here are also in agreement with the theory of polyp-cancer sequence [8, 10] and encourage to further more detailed studies on the usefulness of mucosal arginase as a marker of colorectal malignancy.

REFERENCES