Biochemical Changes of Mevalonate Pathway in Human Colorectal Cancer

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Abstract. Background: Alterations in the mevalonate pathway may contribute to malignant cell growth. There are differences in the aetiology, clinical behaviour, pathological and genetic features in cancer of the right versus the left colon. Here, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, farnesylpyrophosphate (FPP) synthase and farnesyltransferase (Ftase) activities were measured in human colorectal cancer (CRC) and normal mucosa in order to evaluate their role as potential markers of malignancy, also in relation to cancer location. Patients and Methods: HMG-CoA reductase, FPP synthase and Ftase activities were determined in CRC and normal mucosa of 90 patients by radiochemical assay. Results: The enzymatic activities were higher in cancer than in normal mucosa. The tumours located at the left side showed higher HMG-CoA reductase activity, whereas the right side tumours showed higher levels of Ftase and FPP synthase activity. Conclusion: The determination of mevalonate pathway enzymes in relation to CRC location may be clinically relevant in designing anticancer targeted therapies.

The mevalonate pathway, in addition to cholesterol, produces a number of end-products known to have an essential role in cell survival and proliferation. Experimental evidence has demonstrated that one of the potential mechanisms that supports colorectal carcinogenesis is the up-regulation of mevalonate-mediated cell growth (1, 2). An enhanced request for cholesterol by cancer cells produces biochemical changes in the cholesterol biosynthetic process, determining an overexpression of the key enzymes of this biochemical system.

High levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (3-9), farnesylpyrophosphate (FPP) synthase (10-12) and farnesyltransferase (Ftase) (13-16) have been found in different human cancers. HMG-CoA reductase catalyses the conversion of HMG-CoA into mevalonate. An important intermediate of the mevalonate pathway is FPP, an unsaturated carbon chain produced by FPP synthase. FPP is a precursor of several products of the mevalonate pathway, such as cholesterol, heme A, dolichols and ubiquinones. Moreover, FPP is essential for the activation of a variety of intracellular proteins. In this activation step, the farnesyl moieties are coupled to the protein, resulting in a farnesylated protein. This reaction is catalysed by Ftase. Farnesylation is central to the intracellular localization and proper function of several proteins such as Ras, nuclear lamins, transducin γ, rhodopsin kinase, Rho and all of the remaining heterotrimeric G proteins and small G proteins. These prenylated proteins have been most extensively studied and are involved in important functions such as cell growth and differentiation, signal transduction and cytoskeletal organization. Since mutated Ras proteins have been detected in approximately 30%-50% of human cancers, aberrant Ras function is thought to play a role in carcinogenesis (17). Therefore, therapeutic strategies targeted at the three enzymatic activities of the mevalonate pathway are in clinical evaluation (18).

Previously, we showed that HMG-CoA reductase activity was higher in human colon cancers not expressing Low Density Lipoprotein (LDL) receptor (8) and that enhanced activity of HMG-CoA reductase in colon cancer was associated with the location of the tumour (9). In a previous study, we were able to detect FPP synthase activity in human colon cancer; higher significant levels of FPP synthase activity and its mRNA were detected in cancer than in normal mucosa (12). In addition, we found an increased Ftase activity in human colorectal cancer, showing differences in Ftase activity in relation to histological grading, tumour location and K-ras mutation (16).

On the basis of these data, it appears clear that alterations in biosynthetic processes of the mevalonate pathway and in the levels of enzyme products participating in this biochemical system may contribute to the cell growth...
advantage acquired during the carcinogenic process and to the development of malignancy.

Moreover, different segments in the large bowel may have different susceptibilities to neoplastic transformation. Several studies have indicated that there are differences in the aetiology, clinical behaviour, pathological features and genetic abnormalities in cancer of the right colon versus the left colorectum (19). This evidence supports the theory that the oncogenesis of the left, right- and rectum-sided cancers may involve, at least partially, different mechanisms (19, 20).

In this study the HMG-CoA reductase, FPP synthase and Ftase activities were measured in human colorectal cancer (CRC) and normal mucosa, in order to evaluate the role of these enzymes as potential markers of malignancy, also in relation to cancer location. Moreover, the K-ras mutation status of patients was also investigated.

Patients and Methods

Ninety CRC patients (48 males and 42 females, mean age 64.2±11.2 years) were enrolled in the study. Colorectal mucosa and cancer were obtained from each of them. Specimens were taken within one hour after surgical procedure and stored at –80°C until assayed.

For each patient, information on clinical characteristics and histopathological features was recorded.

HMG-CoA reductase activity assay. HMG-CoA reductase activity was measured as [14C]-mevalonolactone formed in resuspended microsomal pellets, by radiochemical assay using DL-3-hydroxy-3-methyl-[3-14C]-glutaryl-coenzyme A ([14C]-HMG-CoA) as substrate. The charcoal microsomes were prepared as previously described (8). Microsomal HMG-CoA reductase activity was assayed in two sequential steps: a) a pre-incubation period, during which microsomes were incubated in the presence of Escherichia coli alkaline phosphatase, in order to prime the enzyme, and b) a subsequent incubation period, during which the HMG-CoA reductase activity was measured. Both phases have previously been described extensively (8). HMG-CoA reductase activity was expressed as picomoles of [14C]-mevalonate formed per minute per milligram of microsomal proteins (pmol/min/mg prot). Normal liver tissue was used as the positive control in each experiment.

Farnesyl diposphate synthase assay. FPP synthase assay was carried out as previously described (12). Briefly, FPP synthase was assayed in 150 µl containing 25 mM HEPES, pH=7, 2 mM MgCl2, 1 mM dithiothreitol, 5 mM KF, 1% n-octyl-β-D-glucopyranoside, 3.3 µM [4-14C] IPP (18 Ci/mmol), 3 µM unlabelled IPP and 20 µM geranyl dithiothreitol, 5 mM KF, 1% n-octyl-β-D-glucopyranoside, 3.3 µM [4-14C] IPP (18 Ci/mmol), 3 µM unlabelled IPP and 20 µM geranyl dithiothreitol. The reaction mixture was incubated for 10 min at 37°C and centrifuged at 10,000 x g for 10 min at 4°C. The supernatants were centrifuged at 100,000 x g for 1 h at 4°C. To 20 µl of [3H] farnesyl diphosphate solution, were added 20 µl of 50 mM Hepes, 30 mM MgCl2, 20 mM KCl, 5 mM DTT and 0.01% of Triton X-100, 20 µl of biotin lamin B and 40 µl of cytosol fraction containing 25 µg of total proteins. After incubation for 1 h at 37°C, the reaction mixture was stopped by the addition of 150 µl of stop solution containing streptavidin-linked beads. Parallel samples were assayed to detect the total and the aspecific radioactivity. In all experiments, enzyme assays were carried out in duplicate. The variation coefficient percentages (CV%) of the intra- and inter-assay were 3% and 4% respectively. Ftase activity was expressed as the picomoles of [3H] farnesyl-biotin lamin B formed per 1 h per mg of cytosol protein (pmol/h/mg prot).

K-ras mutation analysis. Tissue DNA was extracted using TRI-Reagent (Mol. Res. Centre Inc., Cincinnati, OH, USA) following the manufacturer’s instruction. K-ras mutation was assayed by polymerase chain reaction (PCR), using sense primer (5'-TGAAATATAAATCCTGTGTTAGTGGACCT-3') and antisense primer (5'-CAAAGAATGGTGCCCTGGAC-3'), followed by restriction analysis. The conditions of DNA amplification have been previously described (16). PCR amplified samples were digested with the restriction enzyme MvaI (Roche Mol Biochem., Mannheim, Germany) and incubated at 37°C for 3 h. The digested PCR products were electrophoresed through 3% agarose gel containing ethidium bromide for DNA detection. When wild-type DNA is amplified, K-ras primers, a 157 bp product is created containing two restriction sites. This product yields fragments of 29, 114 and 14 bp after enzymatic digestion. When a mutation is present at position 1 or 2 of K-ras codon 12, only one restriction site is formed, which yields digestion products of 143 and 14 bp. Samples were scored positive for K-ras mutations showed a band of 114 bp on the gel.

Results

The clinical and histopathological features of all patients studied are given in Table I.

HMG-CoA reductase, FPP synthase and Ftase activity levels were higher in cancer than in normal mucosa (Table II).
In normal tissue, the HMG-CoA reductase, FPP synthase and Ftase activity levels were not associated with tumour location (data not shown). The tumours located at the left side showed higher HMG-CoA reductase activity ($\chi^2$-test, $p=0.02$), whereas the tumours located at the right side showed higher levels of Ftase and FPP synthase activity ($\chi^2$-test, $p=0.01$ and $p=0.06$, respectively).

K-ras mutation was detected in 28 out of 90 patients (31% of cases). As seen in Table III, higher FPP synthase and Ftase activity levels were measured in K-ras-positive patients compared with patients without K-ras mutation. No statistically significant differences in HMG-CoA reductase activity were detected between K-ras-positive and -negative patients.

**Discussion**

The up-regulation of the enzymes of the mevalonate pathway examined in this study is due to an increased cholesterol demand by the neoplastic cells of these tumours. Recently, the influence exerted by isoprenoid on the growth of human colonic adenocarcinoma cells has been demonstrated, suggesting that, in rapidly proliferating tumours, the production of isoprenoid intermediates is intrinsically enhanced (1, 21). Previously, we have shown that HMG-CoA reductase activity was higher in human colon cancer not expressing low density lipoprotein receptor, and these features were associated with decreased patient survival (8, 22). In the present study, the HMG-CoA reductase, FPP synthase and Ftase activities were higher in neoplastic samples than in normal ones, in accordance with our previous studies (8, 9, 12, 16). Thus, the involvement of high HMG-CoA reductase activity and high levels of mevalonate-derived metabolites, such as isoprenoid compounds, in the biological aggressiveness of cancer is evident.

The differences in HMG-CoA reductase, FPP synthase and Ftase activities among the left, right colon and rectal locations detected in this study underline regional variability in the neoplastic cell proliferation of the large bowel. Several studies have indicated that there are differences in the aetiology, clinical behaviour, pathological features and genetic abnormalities in cancer of the right colon versus the left side.
left colorectum (19). This evidence supports the theory that the oncogenesis of the left-, right- and rectum-sided cancers may involve, at least partially, different mechanisms (20). In colon cancer distant metastasis is the major problem, compared to local recurrence in rectal cancer. It is, therefore, reasonable to suggest that the aetiological factors and molecular basis may differ between colon and rectal cancer (23). Environmental factors that could potentially favour the development of right- or left-sided colon tumours include diet, physical activity, smoking, cholecystectomy, chemopreventive agents, reproductive and hormonal status (20). Previously, we demonstrated that growth factors, such as EGF and TGFα, are expressed more in the left colon than in the right one (24). Different segments in the large bowel may also have different susceptibilities to neoplastic transformation. Kinetic changes are more marked in the left colon than in the right colon, as shown by the labelling index of the upper crypt regions (25). Molecular features are responsible for determining tumour phenotype, and genetic and biochemical factors may be linked to the anatomical site of the tumour. Moreover, distal lesions show a higher incidence of chromosomal aberrations and hyperploidy (26), whereas proximal colonic tumours are often associated with normal karyotypes and characterized by diploid DNA content.

The K-ras mutation has been demonstrated to be linked to malignancy in 20-30% colorectal tumours (17). Since FPP synthase supplies the substrate for the farnesylation by Ftase enzyme of a wide number of proteins implicated as potential growth regulators, including gene products of the K-ras oncogene, the detection of higher Ftase and FPP synthase activities in K-ras-positive colon cancers in this study supports the hypothesis of a cellular adaptation in response to K-ras-induced transformation. This is in line with our previous observations of an association between farnesylation of Ras protein and cancer (13, 16).

The demonstration of the up-regulation of isoprenoid pathway enzymes in colorectal cancer clarifies our understanding of colon tumorigenesis, and the association with tumour location also contributes to the development of therapeutic targets linked to the anatomical site of neoplasia. Our data, thus, suggest that stratification of patients with colorectal cancer into right- and left-sided subsets may be important in optimal patient selection for HMG-CoA reductase-, FPP synthase- and Ftase-inhibitor therapy and for subsequent assessment of objective therapeutic response.

Acknowledgements

The authors would like to thank Ms Benedetta D’Attoma for her excellent technical assistance.

This work is supported by a Grant of Ministero della Salute n° ICS-160.2/RF99.65, Italy.

References


Received March 1, 2005
Accepted June 1, 2005