Tissue Expression of Glycated Apolipoprotein B in Colorectal Adenoma and Cancer

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Abstract. *Background: Myocardial infarction and colorectal cancer are associated at the population level and in autoptic studies. Glycated apolipoprotein B (apoB) is a risk factor for the development of myocardial infarction. The association of glycated apoB with dysplastic and neoplastic colorectal tissue was investigated. Materials and Methods: Forty-eight consecutive surgical specimens, 26 colorectal adenomas and 22 colorectal carcinomas, retrieved from the archives of the Pathologic Anatomy Department of our institution, were examined. The tissue content of glycated apoB was determined using a monoclonal antibody. Results: Glycated apoB was detected in 27% of the adenomas and 45% of the cancer tissue, but only in 18% of the normal tissue near the cancer site. Conclusion: Glycated apoB is associated with dysplastic and even more so with neoplastic cancer tissue.*

Apolipoprotein B (apoB) is the main apoprotein of low density lipoprotein (LDL), the amphoteric complex that transfers cholesterol from the liver to peripheral tissues (1). ApoB has a critical function in the uptake of cholesterol by the cell, a receptor-mediated process of endocytosis (2). In fact, apoB contains the binding site for the LDL receptor expressed on the cell surface (3).

LDL can undergo glycation, with glucose forming a covalent bond with the lysine of apoB (4). Glycation is a post translational modification involving intracellular and extracellular proteins (5). The process consists of the reaction of glucose with susceptible amino groups, often a lysine residue on the protein (5). Glycation happens through several stages with different kinetics, in the absence of any enzymatic action. Only later stages of this reaction are irreversible and lead to the formation of advanced glycation end products (AGEs), molecules which can only accumulate in the organism (6). A large body of evidence shows that AGEs are involved in the pathogenesis of many degenerative diseases (6-8). Moreover, products of glycation accumulate in human cancer tissue (9, 10), and the receptor for advanced glycation end products is implicated in tumor cell proliferation and migration (11).

Plasma glucose concentration is the principal factor affecting the rate of glycation (12). In conditions of persisting hyperglycemia and diabetes, many blood proteins undergo glycation (13).

Glycated apoB has a short life in the blood stream, 3-5 days (14) in comparison to other glycated blood proteins of common clinical practice, such as fructosamine (2-3 weeks) (15) and glycated hemoglobin (2-3 months) (16). Therefore, variation of serum glycated apoB can be used as a short-term marker of glycemic control (14).

Glycation of LDL apoB involves epitopes close to its receptor-binding site, suggesting that a change in this portion can affect recognition by LDL receptors (17). In fact, during *in vivo* turnover studies and in tissue culture, it has been observed that glycated LDL is not cleared by LDL receptors (17, 18).

Glycation of LDL increases lipid peroxidation (19, 20) enhancing LDL atherogenicity through recognition and internalization of oxidized apoB adducts by macrophage scavenger receptors and the formation of foam cells (21, 22). Macrophage uptake of glycated LDL is greater than that of native LDL (23).

A higher concentration of glycated LDL apoB has been detected in the sera of diabetic patients, although it is found also in the sera of healthy non-diabetics (24), contributing to atherogenic risk even in non-diabetic people (25).

A recent cohort study showed that glycated apoB is an important risk factor for myocardial infarction (26). Autoptic studies and epidemiological evidence have shown that

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Table I. *Clinicopathological characteristics of patients with carcinoma.*

Characteristic	
Age, median (range)	69 (44-80 years)
Gender, male/female	15/7
Tumor site	
Left colon	9
Rectum	13
Tumor grade	
G1	1
G2	9
G3	12
Dukes' stage	
B	11
C	10
D	1

Table II. *Clinicopathological characteristics of patients with adenoma.*

Characteristic	
Age, median (range)	66 (51-74 years)
Gender, male/female	18/8
Tumor site	
Left colon	24
Rectum	2
Dysplasia	
mild	0
moderate	6
severe	20

myocardial infarction and colorectal cancer are associated (27, 28), and also share some risk factors, such as hypertriglyceridemia (29), therefore it is reasonable to hypothesize a common pathogenetic mechanism.

The aim of this study was to evaluate the presence of glycated apoB in tissue sections from patients with confirmed colorectal adenomatous polyps and cancer, and in nearby normal colorectal mucosa, to test the hypothesis that glycated apoB is associated more with the dysplastic and neoplastic mucosa than with the normal mucosa.

Materials and Methods

Samples. A consecutive series of surgical specimens of formalin fixed, paraffin embedded colorectal adenomas and carcinomas collected in one year were retrieved from the archives of the Department of Pathology of our institution. All the tissue blocks were re-cut and reviewed by the pathology team for confirmation of the diagnosis and adequacy of the material. After the evaluation, 26 colorectal adenomas and 22 colorectal carcinomas were selected for the study.

Serial sections (4 μm thick) were cut and used for hematoxylin-eosin staining (to ensure the presence of normal, dysplastic and neoplastic epithelium) and for immunohistochemical detection.

Written informed consent was obtained from all the participants.

Table III. *Positivity for glycated apoB in normal tissue, adenoma and cancer.*

Total	Glycated apoB (+)	% shown*	Histological features of sample
22	4	18	Normal mucosa surrounding cancer
26	7	27	Adenoma
22	10	45	Carcinoma

*Chi-square for trend, $p<0.05$.

Table IV. *Mc. Nemar test, Chi-square, for matched pairs, p<0.05.*

	Normal mucosa	
	Glycated apoB (+)	Glycated apoB (–)
Carcinoma		
Glycated apoB (+)	4 (40%)	6 (60%)
Glycated apoB (–)	0	12 (100%)

Immunohistochemistry detection. In our experience, there is no specific antibody for glycated apoB commercially available for immunohistochemical assays. Therefore the monoclonal antibody (MAb) ES-12, specific for glycated apoB in Western blot, was used in this analysis, after appropriate technical improvement. MAb ES-12 is commercially produced by Exocell (Exocell Inc., Philadelphia, Pennsylvania, USA); the recommended dilutions of the product are 1:10 to 1:100, corresponding to antibody concentrations in the range of 1 and 10 μg/ ml. In order to determine the highest dilution of antiserum resulting in optimal specific stain with the least background, 4 μm tissue sections were incubated with MAb ES-12 at dilutions from 1:10 to 1:1000 in phosphate-buffered saline (PBS), pH 7. Each dilution was tested by incubating samples for 30 min at RT or overnight at 4˚C, with or without previous antigenic retrieval in a microwave oven. A standard peroxidase-conjugated polymer, which carries antibodies to rabbit and mouse immunoglobulin (Envision, Dako, Glostrup, Denmark) was used according to the manufacturer's recommendations to visualize the bound antibodies. For standardization of the various steps in the immunostaining process, overall immunohistochemical detection was performed in an automatic autostainer (Dako). Negative controls included omission of MAb ES-12 substituted either by the non-immune mouse serum at the same protein concentration as the primary antibody, or by irrelevant mouse antibody. The working dilution for ES-12 was found to be 1:800, without previous antigenic retrieval.

The immunohistochemical evaluation was performed on each whole section, avoiding marginal and necrotic areas. The sections were considered positive when at least 5% of the tumor cells had a sharp membranous/cytoplasmic stain above the background level.

Statistical analysis. The association of tissue positivity for glycated apoB with colorectal dysplasia and cancer were analized using the Chi-square test for trend and the Mc. Nemar test, Chi-square test for matched pairs.

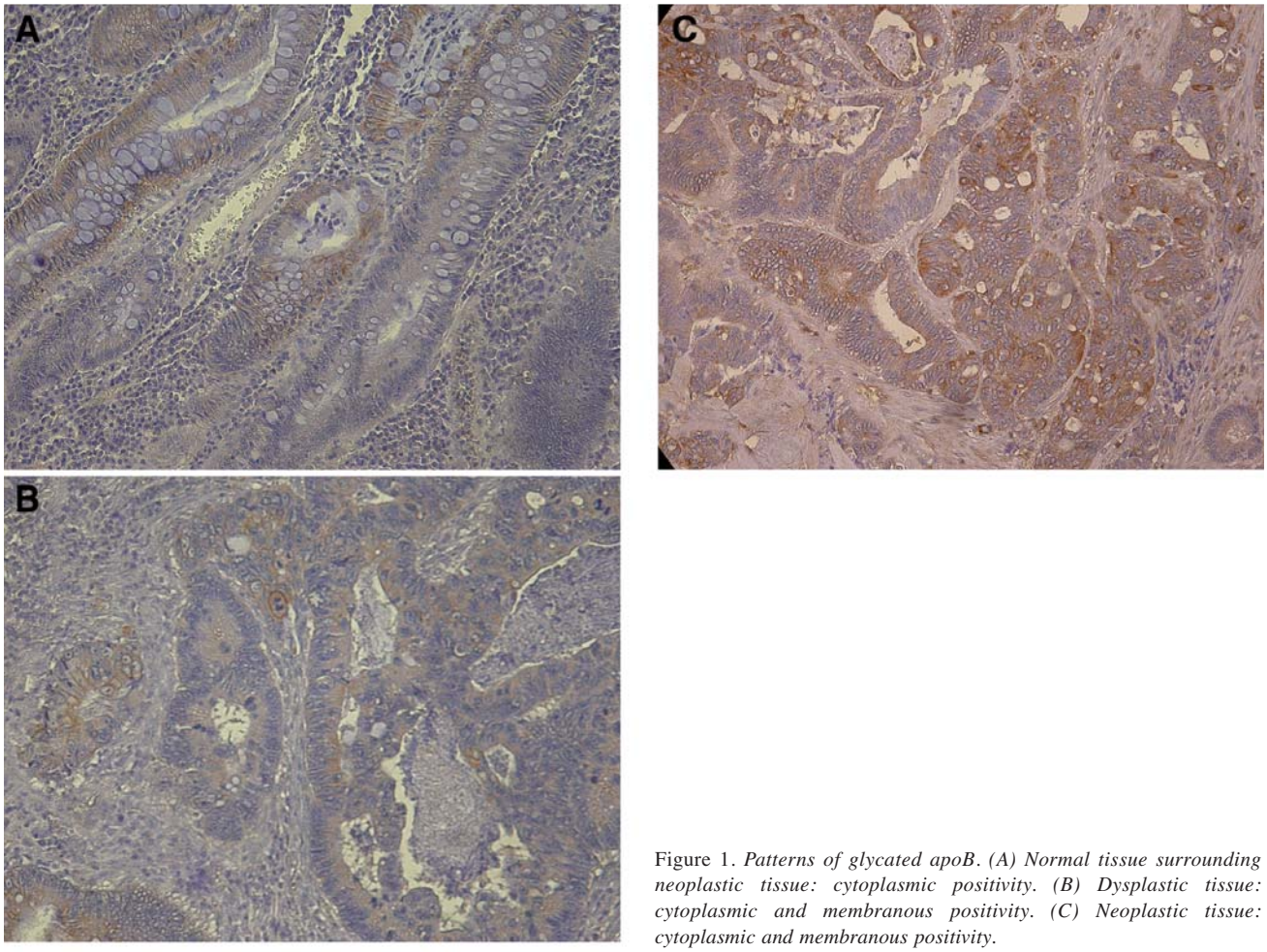

Figure 1. *Patterns of glycated apoB. (A) Normal tissue surrounding neoplastic tissue: cytoplasmic positivity. (B) Dysplastic tissue: cytoplasmic and membranous positivity. (C) Neoplastic tissue: cytoplasmic and membranous positivity.*

Results

All the adenomas had a diameter larger than 1 cm and a tubulo-villous morphology and all the carcinomas were histologically adenocarcinoma. The clinicopathological characteristics of the patients are reported in Tables I and II. Monoclonal antibody ES-12 showed membranous and cytoplasmic reactivity only in epithelial cells. No reactivity was apparent in the negative control slides. There was moderate positivity for glycated apoB at the membrane level and diffuse in the cytoplasm in focal areas in 7 out of the 26 adenomas (27%), in 10 out of the 22 carcinomas (45%) and in 4 out of the 22 samples of normal mucosa surrounding the carcinomas (18%). Figure 1 A-C shows the pattern of positivity of samples for glycated apoB in the normal mucosa surrounding neoplastic tissue, in high-grade dysplastic and in neoplastic tissue, respectively. The proportion of samples positive for glycated apoB from the normal mucosa, to adenoma and carcinoma showed that the expression of glycated apoB increased with the degree of tissue transformation ($p<0.05$) (Table III).

Furthermore, the expression of glycated apoB was associated with the pathological tissue *versus* the matched normal tissue near the cancer site (Chi-square test, $p<0.05$) in the subjects with cancer (Table IV).

Discussion

The expression of glycated apoB increased from the normal epithelium around the cancer site (18%), to the colorectal adenomas (27%) and to the carcinomas (45%).

As previously noted, the sugar covalent modification of apoB affects sequences of the binding site to the receptor of LDL. Therefore, under physiological conditions, glycated LDL should have difficulty in entering the cells. However glycation products accumulate in tumor tissues (9, 10), and some glycated molecules such as N^{ε}-(carboxymethyl)lysine are considered biomarkers of cancer (9). In the arterial wall

glycated apoB interacts with macrophages, which have receptors for glycated, oxidized or glyco-oxidized lipoproteins (23), promoting inflammation and the release of cytokines (23).

Recent data have shown that tumor cells have phagocytic activity which is even more apparent if they are metastatic (30). This feature is considered the greatest expression of the energetic greed of neoplastic cells (31, 32). It can be supposed that malignant colorectal cells act like macrophages, and like the macrophages of the arterial wall in atherosclerosis (associated with colorectal cancer) take up glycated apoB through a phagocytic process. Moreover, cannibal cancer cells 'eat' everything without selecting the consumed substances (30), and this behaviour makes them more similar to microorganisms than to macrophages.

Thus, a possible future utilization of this protein as carrier of therapy can be suggested. The presence of glycated apoB in the normal mucosa surrounding the cancer site may hint that these cells are 'pseudo-normal', and are involved in the degenerative process of malignancy because of the close proximity to the neoplastic tissue. The level of glycated apoB positivity of the normal mucosa being lower than that in dysplastic and neoplastic tissue could mean that the expression of glycated apoB increases with the grade of dysplasia. However, from the findings of this study it seems that glycated lipoprotein enters the cancer cells, and enters more if the cell is less differentiated.

Cancer cells require larger quantities of cholesterol to support faster cell cycles and higher rates of replication than normal cells (33). We have previously reported the decrease of LDL receptor expression in neoplastic colorectal tissue, that has unfavourable prognostic value, predicting a shorter survival for LDL receptor-negative patients (34). It is possible that neoplastic cells inhibit the expression of the LDL receptor as they do not need it, because they obtain cholesterol by consuming glycated apoB. This would not be surprising, since cancer cells shift from ordered, regulated behaviour, to a state of non-organized activities.

In conclusion, our studies indicate that glycated ApoB is associated with dysplastic and even more with neoplastic cancer tissue. However, more evidence is needed to confirm the present findings, and to support the hypothesis that glycated apoB could be involved in the multifactorial pathogenesis of colorectal cancer.

Conflict of Interest

None to declare

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