The importance of acute-phase proteins as markers in cancers

Znaczenie białek ostrej fazy jako markerów nowotworowych

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Abstract

Acute-phase proteins (APPs) form a group of heterogeneous proteins whose synthesis takes place in the liver. This involves mediators such as cytokines and constitutes a response to acute inflammations, bacterial infections, tumours, and autoimmune diseases. C-reactive protein is among the most important APPs. It participates in cancerous processes, especially in colon and rectal cancer. Another APP is haptoglobin. This glycoprotein prevents iron loss and kidney damage, and is associated with diagnostic neoplastic progression connected with fucosylation. Fucoso–haptoglobin is a highly effective marker for prognosis because an increased concentration of this complex is indicative of the distant metastases amount. Another APP is acidic α-glycoprotein (AGP), which increases to a serious level during acute inflammations and tumour-promoting processes. Its protective role involves platelet aggregation inhibition and chemotactic activity. The purpose of this study was to review the role of selected acute phase proteins in cancer promotion and progression.

Streszczenie

Białka ostrej fazy (APP) stanowią grupę białek, których synteza zachodzi w wątrobie z udziałem cytokin jako odpowiedź na ostre stany zapalne, infekcje bakteryjne, nowotwory i choroby autoimmunologiczne. Białko C-reaktywne to jedno z najważniejszych APP. Bierze ono udział w procesach nowotworowych, zwłaszcza w przypadku raka jelita grubego. Kolejnym przykładem APP jest haptoglobina. Ta glikoproteina zapobiega utracie żelaza i uszkodzeniom nerek, a także wiąże się z diagnostyką postępu nowotworu powiązaną z fukozylacją. Fukozo–haptoglobina jest niezwykle efektywnym markerem prognozującym, ponieważ podwyższone stężenie tego kompleksu świadczy o obecności odległych przerzutów. Kolejnym przykładem APP jest kwaśna α-glikoproteina (AGP), której stężenie bardzo wzrasta podczas ostrych stanów zapalnych oraz promowania nowotworów. Jej protekcyjna rola obejmuje inhibicję agregacji płytek krwi i chemotaktyczną aktywność. Celem tego artykułu jest przegląd wybranych białek ostrej fazy i ich funkcji w promowaniu oraz postępie nowotworu.

Introduction

Acute-phase proteins (APPs) are heterogeneous proteins that differ in their physicochemical properties, biological functions, plasma concentration, and the dynamic of modifications of concentrations during inflammation [1]. The synthesis of those proteins occurs as a response to homeostasis disorders triggered by acute inflammations, bacterial infections, tumours, and autoimmune diseases. In 1951, Miller *et al.* [2, 3] demonstrated that the liver is the paramount location of APP synthesis. Acute-phase proteins constrain the spread of the inflammatory process and remove any related aftereffects. APPs contribute thereby to restoring homeostasis in the organism. Their operation consists of complementing system activation, providing non-specific reactions connected with opsonization and agglutination, and containing tissue injuries whether through bacteria or lysosomal enzymes originating from phagocytes or increased chemotactic activity. Furthermore, APPs influence not only the immunological system but also the secretion of certain hormones such as glycosteroids and ACTH [4]. The purpose of this study is to review selected acute-phase proteins as markers in cancers.

The influence of cytokines on acute-phase protein synthesis

There are several cytokines responsible for the induction of APP synthesis. This group includes interleukin-6 (IL-6), interleukin-1 (IL-1), and tumour necrosis factors (TNF) α (TNF-α) and TNF-β. The strongest APP activator is IL-6. However, IL-1 and TNF stimulate fibroblasts, endothelial cells, and keratinocytes to IL-6 synthesis, and they enhance its biological activity [3, 5]. Moreover, IL-6 impacts the phosphorylation of the NF-IL6 transcription factor, which is transported to the cell nucleus because of the modification. There, NF-IL6 subsequently activates gene transcription for APP [5]. On the other hand, IL-1 and TNF phosphorylate the NF-κB transcription factor inhibitor, causing the release of NF-κB and gene activation in the cell nucleus [5].

The importance of cytokines in cancerous transformation

The cells which submitted to neoplastic transformation produce and release a series of cytokines into the circulation, with auto- and paracrine effects. The unbound cytokines are of great value in cancerous cell proliferation and angiogenesis, as well as in the mechanisms leading to an invasive phenotype elicitation and the formation of distant metastases. What is more, they also attend a generation of chemo- and radio-resistance.

TNF-α is produced and released into the circulation. For a long time, it is the main promoter in the development of cancer. Additionally, it induces an expression of NF-κB-dependent genes, anti-apoptotic agents, proinflammatory cytokines, chemokines, adhesion molecules, and metalloproteinases. In 2008 Sethi *et al.* [6] recognized TNF-α as a factor promoting the expansion of TNF-α-dependent cancers. The study showed that there is a connection between an increased concentration of TNF-α and deprivation of hormonal dependence, marasmus development, bad prognosis, neoangiogenesis, tumour progression, and advance of metastases (Table 1).

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein discovered in 1930 by Tilletto and Francis [7] in serum from patients with chronic bacterial pneumo-

Table 1. Classification of acute-phase proteins (APPs)

Classification by the following		
Protein division	**Positive proteins**	**Negative proteins**	**Neutral proteins**
Examples	– C-reactive protein – α1-Antitrypsin – Haptoglobin – Hepcidin – Ceruloplasmin – Plasminogen – Fibrinogen – Ferritin – Procalcitonin	– Transferrin – Prealbumin – Albumin – Inter-α-antitrypsin – α1-Lipoprotein – Antithrombin	– α2-Macroglobulin – Prothrombin – Serosal amyloid P
Classification by increase in degree of concentration		
Protein division	**Proteins with 100–1000-times increased concentration**	**Proteins with 50-times increased concentration**	**Proteins with 2–3-times increased concentration**
Examples	– C-reactive protein – Serosal component of amyloid A	– ceruloplasmin – compounds of fixator C3 and C4 – α1-inhibitor of proteins – α2-antiplasmin – C1-inactivator	– α1-Antichymotrypsin – α1-Acidic – Glycoprotein – Haptoglobin – α1-Antitrypsin – Ferritin – Fibrinogen
Classification by kinetics of changes		
Protein division	**First-line proteins[1]**	**Second-line proteins[2]**
Examples	– Protein CRP – α1-Antichymotrypsin – Erosal component of amyloid A	– Interleukin-6 – Interleukin-8

[1]*First-line proteins – their concentration increases at the earliest after 6–8 h due to the incentive actuation that initiates the acute phase reaction. It achieves highest values after 24–48 h and becomes normalized within several hours.* [2]*Second-line proteins – their concentration increases after 24–48 h, reaches its highest in 72 h, and they are eliminated after several days, and only after this time do their concentrations become normalized.*
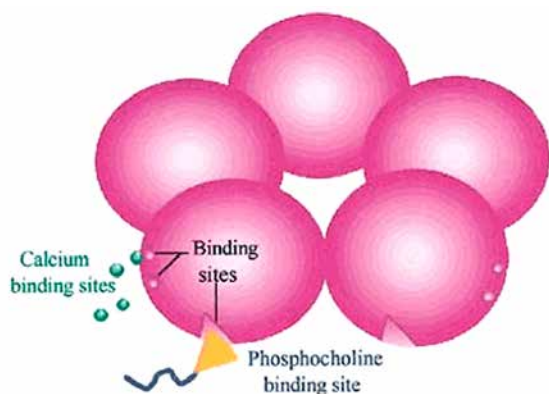
Figure 1. Pentameric structure of C-reactive protein

nia caused by *Streptococcus pneumoniae*. CRP has a cyclic pentamer structure with a molecular weight of 120 kDa (Figure 1). This glycoprotein belongs to the pentaxim family of ligands-binding proteins in calcium ion-dependent reactions. Each of the subunits of the C-reactive protein is made up of 206 amino acids, which are bound together by non-covalent bonds [8]. The synthesis of C-reactive protein takes place mainly in the liver as a response to the pro-inflammatory factors IL-1 and IL-6. Furthermore, C-reactive protein is one of the most important acute-phase proteins, whose concentration in the blood might increase up to 1000 times in 24-72 h during inflammation [9]. CRP also has a vital function in the pathophysiology of malignant tumours, especially in colorectal and rectum cancer.

Heikkilä *et al.* [10] showed in their study that changes in CRP concentration depend on the size of the tumour and the stage of the neoplastic process. In the case of nipple cancer, elevated CRP concentrations were observed only in advanced stages, especially in the presence of metastases [11]. In addition, in the group of patients with colorectal cancer qualifying for surgical treatment, it has been proven that elevated CRP level reduces the number of lymphocytes in peripheral blood.

In 33% of patients with CRP > 8.0 mg/l, a lower percentage of lymphocytes was found, as well as lymph nodes involved in the neoplastic process, vascular infiltration, and liver metastases [10]. Furthermore, in the group of patients with elevated CRP, the percentage of people surviving 2 years after surgery was 47%, while in patients with reduced CRP, the figure rose to as much as 90% [12]. Similar results were presented by Shrotriya *et al.* [13], showing that a CRP level > 10.0 mg/l prior to surgery is associated with the likelihood of shorter overall survival in patients after tumour resection, and it provides an unfavourable prognostic factor in patients with colorectal cancer. In contrast, Son *et al.* [14] reported that patients with CRP > 10.0 mg/l have increased levels of carcino-

genic embryonic antigen (CEA), which is the marker of choice for colorectal cancer.

Research on the appropriateness of CRP determination as a discriminator in the selection of patients with colorectal cancer for surgical treatment is ongoing. The diagnostic sensitivity of this method is 79%, and the specificity is about 71%. In order to increase the effectiveness of this method, albumin concentration determination is used [11]. Demonstration of quantitative relationships between CRP concentration (> 10.0 mg/l) and albumin concentration (< 35.0 g/l) in cancer patients implies the possibility of calculating a Glasgow Prognostic Score (GPS). The prognostic value of GPS has been shown not only in colorectal and rectal cancer but also in patients with oesophageal, breast, ovarian, and renal cancer, especially in advanced stages of the disease [15].

Haptoglobin

Haptoglobin is an α-glycoprotein composed of 4 protein chains (2 α and 2 β chains), synthesized primarily in hepatocytes and *alveoli pulmonis*. Haptoglobin binds free haemoglobin irreversibly and consequently; this complex is internalized in hepatocytes to prevent iron deprivation with urine and kidney damage. Subsequently, the complex is phagocytized and decomposed into amino acids in the reticuloendothelial system. Moreover, haptoglobin is also important in the pathophysiology of cancer [16].

Haptoglobin contains 4 N-glycosylation sites [17]. Glycosylation is a process controlled by the activity of glycosyltransferases and glycosidases [18]. Adjustments in the expression of these enzymes modify the glycosylation of proteins including haptoglobin, which is used in diagnostics to reflect the progression of cancer [19]. According to Nakano *et al.* [20], the progression of hepatocellular carcinoma (HCC 1) is associated with changes in the expression of glycosyltransferase, including α-1,6-mannosyl glycoprotein 6-β-N-acetylglucosaminyltransferase and with movements in liver structure. In turn, Carlsson *et al.* [19] demonstrated that increased binding of haptoglobin to galectin-1 in patients with breast cancer leads to changes in haptoglobin glycosylation and the intracellular fate of the Hp-haemoglobin complex. A specific variety of glycosylation is fucosylation, which occurs under pathological conditions and is connected with carcinogenesis [21]. What is more, the fucosylated proteins are considered potential tumour markers [22]. It should be noted that in healthy individuals, most haptoglobin molecules do not undergo fucosylation [23], which is associated with a low concentration of fucosyl transferases and guanosine-diphosphate-fucose in the liver. Nakano *et al.* [20] reported that in patients with pancreatic cancer, an increase of fucosylated N-glycans was observed on the serum haptoglobin surface on the basis of lectin-enzyme immunosorbent analysis and

mass spectrometry [20]. Furthermore, fucosylated N-glycans on the surface of haptoglobin can be used as tumour markers for hepatocellular cancer [24], lung cancer [25], and colorectal cancer [26]. Currently, research that aims to identify the characteristic site of fucosylation in haptoglobin is underway.

Some research suggests that protein glycosylation variations are sensitive and specific enough to be used for non-invasive monitoring of liver disease progression, including hepatocellular cancer [27]. The majority of published studies determine the amount of N-glycans after enzymatic deglycosylation through PNGaseF [28]. The main assumption of these studies is that movements in the expression of glycosyltransferases cause a uniform change in specific types of glycans in all glycoproteins. This indicates that a "mean" quantification of many proteins and glycosides would be sufficient to monitor disease processes.

Acidic α-glycoprotein/orosomucoid

Acidic α1-glycoprotein (AGP) is a glycoprotein with a molecular weight of 42 kDa, which consists of about 45% carbohydrates attached as complex N-glycans. Orosomucoid is a highly soluble acute-phase protein first described in 1950 by Schmid [29] and by Weimer et al. [30]. The protein occurs in the form of a single polypeptide chain consisting of 183 amino acids. In the human population, we can identify 2 polymorphic variants: ORM1 and ORM2, depending on the amino acids present in positions 32 and 47. Serious APG levels increase in the course of acute inflammation, infection, and carcinogenesis.

In vivo PG infusion in mice prevents hepatitis and the development of specific shock induced by TNF-α. The protective role of APG is to inhibit platelet aggregation and chemotactic activity. However, the data obtained by Hochepied et al. [31] showed that the protective role of APG only occurs when the concentration of AGP increases rapidly because it is inactivated by inhibitors and binding agents in a short time. The proactive role of AGP before summer exposure to TNF-α has prompted many scientists to address this topic. Research on the possibility of using it as an adjuvant in the use of TNF in tumour therapy is currently underway.

Conclusions

Inflammation fulfils a dominant role in the pathogenesis of various types of cancer, closely related to carcinogenesis and tumour progression (e.g. invasion, migration, and metastasis). This review focused on the prognostic significance of systemic inflammatory markers: CRP, acid 1-glycoprotein, and haptoglobin, in cancer diagnosis. The usage of acute phase proteins as cancer biomarkers has great potential. A more detailed understanding of the pro-inflammatory response in cancer pathogenesis and the identification of specific proteomic expression patterns in cancer-associated acute-phase proteins offers hope for the identification of novel molecular diagnostic markers. Furthermore, it creates the possibility to discover new therapeutic strategies for the treatment of solid and malignant tumours.

Conflict of interest

The authors declare no conflict of interest.

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