Adv Exp Med Biol - Clinical and Experimental Biomedicine (2020) 10: 1–8
https://doi.org/10.1007/5584_2020_506

Published online: 14 March 2020

Chitinase 3-Like 1, Nestin, and Testin Proteins as Novel Biomarkers of Potential Clinical Use in Colorectal Cancer: A Review

Anna Szymańska-Chabowska, Jan Juzwiszyn, Beata Jankowska-Polańska, Wojciech Tański, and Mariusz Chabowski

Abstract

Colorectal cancer is the third most commonly diagnosed cancer in males and the second most common in females. Only 10–20% of patients are diagnosed at the early stage of disease. Recently, the role of novel biomarkers of the neoplastic process in the early detection of colorectal cancer has been widely discussed. In this review, we focused on the three novel biomarkers that are of potential clinical importance in diagnosing and monitoring colorectal cancer. Chitinase 3-like 1 protein, also known as YKL-40, and nestin and testin proteins are produced by colorectal cancer cells. YKL-40 protein is a marker of proliferation, differentiation, and tissue morphogenetic changes. The level of YKL-40 is elevated in about 20% of patients with colorectal cancer. An increased expression of nestin indicates immaturity. It is a marker of angiogenesis in neoplastic processes. Testin protein is a component of cell-cell connections and focal adhesions. The protein is produced in normal human tissues, but not in tumor tissues. Downregulation of testin increases cell motility, spread, and proliferation, and decreases apoptosis. The usefulness and role of these biomarkers, both alone and combined, in the diagnostics of colorectal cancer should be further explored as early cancer detection may substantially improve treatment outcome and patient survival.

Keywords

Biomarkers · Colorectal cancer · Nestin · Testin · YKL-40

A. Szymańska-Chabowska
Department of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Wroclaw, Poland

J. Juzwiszyn
Department of Clinical Nursing, Faculty of Health Science, Wroclaw Medical University, Wroclaw, Poland

B. Jankowska-Polańska
Division of Nursing in Internal Medicine, Department of Clinical Nursing, Faculty of Health Science, Wroclaw Medical University, Wroclaw, Poland

W. Tański
Department of Internal Medicine, Fourth Military Teaching Hospital, Wroclaw, Poland

M. Chabowski (✉)
Department of Clinical Nursing, Faculty of Health Science, Wroclaw Medical University, Wroclaw, Poland

Department of Surgery, Fourth Military Teaching Hospital, Wroclaw, Poland
e-mail: mariusz.chabowski@gmail.com; mariusz.chabowski@umed.wroc.pl

1 Introduction

Colorectal cancer significantly contributes to a reduction in life expectancy. According to data from the Global Burden of Disease Study, the number of deaths due to colorectal cancer has increased from 490,200 in 1990 to 771,100 in 2013, which is over a 57% increase (GBD 2013 Mortality and Causes of Death Collaborators 2015). Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females (Torre et al. 2015). The cancer constitutes a major health care and socioeconomic problem. In 2013, colon and rectum cancers ranked eight as the cause of years of life lost in the developed countries, including Poland. In European countries such as the Netherlands and Sweden, these cancers were classified as the fourth leading cause of years of life lost. A lower survival rate is observed in elderly patients and in those from certain geographical areas such as Eastern Europe (Holleczek et al. 2015), possibly due to poor and delayed diagnosis. At an early stage, colorectal cancer does not manifest specific signs and symptoms, so that it is often diagnosed in advanced stage. Maringe et al. (2013) have reported that only 10–20% of colorectal cancer patients are diagnosed when the cancer is in stage A of Duke's classification (Duke 1932). The difference in survival between high- and low-income countries may be associated with different stages of the disease at the time of diagnosis in respective countries. Thus, the availability of reliable screening tools and biomarkers is of essential importance for the diagnostics and management of the disease. Recently, the role of novel biomarkers in the neoplastic process and early detection of colorectal cancer has been widely discussed (Corbo et al. 2017; Das et al. 2016). In this review we focused on three novel biomarkers that have been under scrutiny as being of potential clinical importance in colorectal cancer diagnosis and monitoring. These biomarkers are chitinase 3-like 1 protein, also known as YKL-40, and nestin and testin proteins; all produced by colorectal cancer cells.

2 Biomarkers and Risk Factors Associated with Colorectal Cancer

An unhealthy diet, including consumption of processed meat products and alcohol, obesity, sedentary lifestyle, and smoking, can all contribute to the development of colorectal cancer (González et al. 2017; Friedenreich et al. 2016; Ferrari et al. 2007). Mitigating the effects of such modifiable risk factors may reduce the level of colorectal cancer morbidity (Johnson 2017; Doleman et al. 2016; Botteri et al. 2008). Patients at high risk are advised to participate in screening programs. Such programs are, however, of rather low sensitivity and specificity for the detection of colorectal cancer and for the avoidance of the cancer high invasiveness. Patients tend to avoid tests such as sigmoidoscopy or colonoscopy, despite the evidence of efficacy of these tests in the diagnosis of colorectal cancer. Therefore, the identification of new blood-derived biomarkers, which patients would be more complaint with, or noninvasive molecular tests would greatly benefit treatment outcome and survival.

The term tumor biomarker was coined in 1988 as a medical subject heading, and it was defined as "molecular products metabolized and secreted by neoplastic tissue and characterized biochemically in cells or body fluids". Biomarkers are indicators of tumor stage and grade and are useful for monitoring the response to treatment and prognosticate recurrence. Biomarkers are represented by a host of molecules such hormones, antigens, amino acids, nucleic acids, enzymes, polyamines, and specific cell membrane proteins and lipids (Goossens et al. 2015).

Currently, there are only two protein biomarkers detectable in blood serum, which are approved for clinical use in colorectal cancer. The carcinoembryonic antigen (CEA) is one of the two. Preoperative elevation of CEA is associated with reduced overall survival. A lack of postoperative normalization of CEA level may indicate an incomplete tumor resection and thus portends a recurrence. CEA monitoring helps identify

patients with metastases, which particularly concerns the liver (Goldstein and Mitchell 2005; Duffy 2001). A disadvantage of CEA is that it lacks sensitivity in the early stages of colorectal cancer. It can also be increased in patients without cancer, being secreted in response to various inflammatory processes, e.g., hepatitis, inflammatory bowel disease, pancreatitis, chronic obstructive pulmonary disease, or oxidative stress in diabetic patients and smokers (Hasan and Mohieldein 2015; Tanaka et al. 2010; Fukuda et al. 1998).

Another biomarker widely used in colorectal cancer detection is fecal hemoglobin (f-Hb), which is usually detected using an immunochemical test. The test does not require dietary or medication restrictions. One or two stool samples are enough to conduct the test. The overall sensitivity and specificity of FIT in colorectal cancer is 79% and 94%, respectively, although these parameters may change due to inter-lab differences in cutoff values used for positive FIT results. The optimum cutoff value is still debatable (Lee et al. 2014; Tanaka et al. 2010). The use of FIT as a screening tool has been shown to decrease mortality associated with colorectal cancer (Chiu et al. 2015; Giorgi et al. 2015). The disadvantage of the test is its lower unsatisfactory sensitivity for detection of early stage cancers. To this end, the search for novel more accurate biomarkers continues (Chiu et al. 2013).

3 Chitinase 3-Like 1 (YKL-40) Protein in the Diagnosis of Colorectal Cancer

Chitinase 3-like 1 (CHI3L1), also known as YKL-40 protein, is a human cartilage glycoprotein-39 (HC-gp-39) that was first described in 1992 as a protein secreted by the MG-63 human osteosarcoma cell line (Johansen et al. 1992). In addition to cancer cells, YKL-40 also is secreted by inflammatory and stem cells. In normal tissues, high YKL-40 expression has been noticed in embryos and fetuses where the processes of proliferation, differentiation, and tissue morphogenetic changes are highly active (Johansen et al. 2007). The blood content of YKL-40 in healthy adults is fairly stable, with the median of 43 μg/L as determined in a study by Johansen et al. (2008) and 40 μg/L in the Danish NORDIC VII study that included 3130 subjects (Tarpgaard et al. 2014), with increasing levels with age. An elevated plasma level of YKL-40 has reported in patients with rheumatoid arthritis, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, asthma, liver fibrosis, severe infections, as well as in patients with cancers inter alia breast, lung, prostate, colorectal, and gastric cancers (Schultz and Johansen 2010; Roslind and Johansen 2009). Such wide spectrum of different pathologies where YKL-40 increases indicates a lack of specificity. Therefore, comorbidities should be taken into account when assessing the diagnostic utility of YKL-40 investigated for a primary disease.

Kawada et al. (2012) have noticed significant differences in plasma content of YKL-40 between control subjects and patients with stage I/II and stage III/IV colorectal cancer. Those authors have also reported increased YKL-40 mRNA expression in cancer tissue when compared with normal adjacent tissues. Therefore, elevated YKL-40 content may be related to a more aggressive phenotype of cancer, having a high metastatic potential. Cintin et al. (2002) have reported that preoperative serum YKL-40 content rises above the age-corrected 95th percentile of healthy volunteers in 19% of patients with colorectal cancer. In a study by Johansen et al. (2015), YKL-40 is elevated in 20% of patients with colorectal cancer, 15% with rectal cancer, 11% with adenoma, 9% with other nonmalignant diseases of the digestive tract, and 8% with no pathological endoscopic findings. Likewise, Tarpgaard et al. (2014) have found that plasma YKL-40 is higher than the upper normal level in 40% of non-resectable metastatic colorectal cancer.

Postoperatively, YKL-40 may decrease in patients who have it within the normal range before surgery, and that may be a positive

prognostic sign. The elevated level of YKL-40 appears to be an independent prognostic of short survival, based on a study including 603 patients who underwent a primary large bowel resection for colorectal cancer (Cintin et al. 1999). In another study, Cintin et al. (2002) have shown that patients who underwent curative tumor resection and had high serum YKL-40 6 months post-surgery were burdened with increased risk of death. In both studies, the YKL-40 content was independent of the serum CEA content. A comparison between YKL-40 and CEA contents performed by Ye et al. (2014) has revealed that YKL-40 is less accurate for diagnosing colorectal cancer, but better for diagnosing tumor recurrence. YKL-40 also appears of help in diagnosing the early-stage colorectal cancer and in monitoring recurrences when combined with CEA.

A high content of YKL-40 is an independent prognostic of poor response to preoperative chemoradiotherapy in locally advanced rectal adenocarcinoma, although the prognostic value of the protein for survival has not been confirmed. It is also worth mentioning that tumor expression of YKL-40, assessed by immunohistochemistry, takes place in 62% of cases in (Senetta et al. 2015). In a study by Johansen et al. (2015), blood content of YKL-40 was higher in patients with colon and rectal cancer than in those with adenoma and other nonmalignant diseases. In patients with colorectal cancer, content of YKL-40 correlated with the stage of cancer, and it was lower in patients with a rectal tumor. Comorbidities are associated with an increase in YKL-40. Thus, the protein is thought to be a good prognostic of colorectal cancer in patients without comorbidities. Ye et al. (2014) have found higher levels of blood YKL-40 in patients with recurrent and metastatic colorectal cancer than in those with the primary diagnosis of colorectal cancer or shortly after surgery. Liu et al. (2014) have confirmed the presence of a link between shorter survival and a high blood YKL-40, but they failed to notice any relationship between progression-free survival and the type of chemotherapy regimen or the histologic type of tumor. In 510 patients with metastatic colorectal cancer in the NORDIC VII study, high pretreatment plasma YKL-40 content is associated with shorter progression-free survival and with overall survival (Tarpgaard et al. 2014).

4 Mechanisms of Action of YKL-40

YKL-40 is produced by various types of restricted cells including colonic epithelial cells and macrophages. The content of this protein is elevated not only in colorectal cancer but also in other bowel diseases such as inflammatory bowel disease, ulcerative colitis, and Crohn's disease (Kamba et al. 2013; Koutroubakis et al. 2003; Vind et al. 2003). Chen et al. (2011) have reported that the expression of YKL-40 is significantly increased in colonic epithelial cells of non-dysplastic mucosa in patients with ulcerative colitis harboring neoplastic lesions when compared with patients without dysplasia and with healthy subjects. A greater expression of YKL-40 concerns more invasive and metastatic tumors. The YKL-40 also has a growth-stimulating effect akin to that of insulin-like growth factor-1, and it stimulates the migration of colonic epithelial cells. Additionally, YKL-40 stimulates the production of interleukin-8 (IL-8) and tumor necrosis factor-α by activation of nuclear factor kappa B (NF-κB) signaling pathway in a human colon cancer cell line (SW480 cells). Other studies confirmed the presence of NF-κB in inflamed intestinal mucosa and a strong association between increased NF-κB activity and colorectal carcinogenesis in the animal model (Popivanova et al. 2008; Rogler et al. 1998). Elevated YKL-40 in epithelial cells may not only suggest inflammation-associated malignant transformation but may also control intestinal inflammation and promote dysplasia in colonic cells (Chen et al. 2011). The findings of Kawada et al. (2012) have shown that overexpression of YKL-40 leads to increased chemotaxis of macrophages and to angiogenesis associated with increased release of IL-8 and monocyte chemoattractant protein-1 (MCP-1) from SW480 cells. Studies in vitro have shown that angiogenic activity of YKL-40 is independent of vascular

endothelial growth factor (VEGF). The effects of YKL-40 on endothelial cell activation may be blocked by silencing S1 expression in the cells or by an anti-YKL-40 antibody (Shao et al. 2009).

5 Nestin in Colorectal Cancer

Nestin is a class VI intermediate filament protein whose expression is upregulated in many kinds of tumor and some other tissues in both experimental models and human samples (Matsuda et al. 2013; Ishiwata et al. 2011). The protein is expressed mainly in neuroepithelial stem cells and is often present in tissues that undergo a repair process. Increased expression of nestin indicates immaturity (Ehrmann et al. 2005; Mokry et al. 2004). Nestin appears a useful marker of microvessel density (MVD) which is a prognostic factor in neoplastic malignancies, including colorectal cancer (Amoh et al. 2005). A significant correlation between MVD and liver metastases shows that tumors' potential to grow, spread, and metastasize is related to the angiogenesis in and around cancer tissue (Choi et al. 1998; Tomisaki et al. 1996). MVD is an important prognostic factor, but the most essential information is provided by the number of newly formed blood vessels because they have leaky and weak basement membranes, which enables the entry of tumor cells into circulation and further spread. In contrast, it is much more difficult for malignant cells to penetrate the endothelial layer of a mature microvessel.

Many commonly used endothelial markers stain and identify both the newly formed small blood vessels and the already existing larger blood vessels. Teranishi et al. (2007), using an animal model, have shown that the CD34-labeled pattern of colorectal cancer blood vessels differs from that of the nestin-labeled pattern. CD34 is detected in the endothelial cells of larger blood vessels with a median diameter of 9.67 μm, whereas nestin is in smaller microvessels of the median diameter of 9.06 μm. In human colorectal cancer tissue, there are significant differences in the median diameter of blood microvessels, ranging from 8.82 to 6.30 μm. Stronger expression of nestin than CD34 also is found in the infiltrating border of a tumor. Further, MVD determined by nestin labeling appears a better prognostic factor for survival than MVD determined by CD34.

Reports on the connection between increased nestin expression and clinical characteristics of colorectal cancer are scarce. Tajima et al. (2014) have demonstrated a patient with an aggressive, undifferentiated carcinoma of the descending colon where there was an overexpression of several proteins including nestin, which prognosticated a poor prognosis. The patient experienced a recurrence 39 days after surgery and died 2 months later. Using clinical samples of colorectal cancer, Li et al. (2015) have shown that nestin is associated with tumorigenesis as its expression is higher in cancer tissue than in normal tissues. These authors further show nestin labeling in the endothelium of small-sized tumor vessels and in stromal cancer cells. Additional in vitro tests show that a knockdown of nestin arrested the cell cycle at S phase and inhibited the proliferation and migration of colorectal cancer cells. Such findings demonstrate that nestin can be used not only as a prognostic marker, but that it also gives hope for the development of a new therapeutic option for cancer patients.

6 Testin Protein in Colorectal Cancer

The testin protein is encoded by a gene which is located at human chromosome 7q31 within the common fragile chromosomal region FRA7G. This locus is susceptible to cancer-associated chromosomal aberrations which may play a role in the oncogenic process (Tatarelli et al. 2000). The protein is a component of cell-cell connections and focal adhesions. It can interact with other types of focal adhesion proteins and connect the actin cytoskeleton to the extracellular matrix. There structures, along with integrin receptors, play a role in cell motility, spreading, proliferation, and apoptosis (Coutts et al. 2003). The protein is produced in normal human tissues, but not in tumor tissues, such as breast, prostate, liver, ovarian cancer, *in utero* glioblastoma, and others (Hu et al. 2015; Yongbin et al. 2014;

Chene et al. 2004). Downregulation of testin can be due to a loss of heterozygosity of the testin gene or by hypermethylation of its promoter. Clinically, a reduced level of testin protein is found in 89% of patients with glioblastoma. Additionally, downregulation of testin associates with a worse outcome and a shorter survival time. It seems a biological plausibility that enhancing testin expression could attenuate the malignant character of cancer cells (Fu et al. 2015. Bai et al. 2014).

In colorectal cancer, Li et al. (2015) have noticed a significantly lower level of testin mRNA and protein expression when compared with adjacent tumor-free tissue samples. Those authors have also found an adverse association between the histological grade, but not TNM stage, of colorectal cancer and the level of testin. Studies *in vitro* have shown that both testin mRNA and protein expression are remarkably reduced in nine colorectal cancer cell lines, but not in the two kinds of normal human colon cells. Further, in vitro overexpression of testin reduces the colony formation efficiency, inhibits cell growth, and increases mRNA and protein expression of pro-apoptotic proteins, whereas the opposite is present when testin is knocked down. The suppressive effect of testin on colorectal cancer cells has also demonstrated in the murine model.

7 Conclusions

The role of biomarker proteins such as YKL-40, nestin, and TES in carcinogenesis is not yet full elucidated. The proteins are associated with cancer aggressiveness and prognosis. A combined assessment of several biomarkers may improve diagnosis and may better prognosticate recurrence and survival. There is a need for further studies to explore the molecular mechanisms underlying the development of colorectal cancer.

Acknowledgments Funded by Wroclaw Medical University grant no. ST.E020.17.050.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval This review article does not contain any studies with human participants or animals performed by any of the authors.

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