

Review article

Pathophysiology of Musine Expression in Colon Tumors

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Abstract

Colorectal carcinomas are among the most common cancers in the world, after skin, lung and breast cancers, and are the third leading cause of cancer-related death. Many different histopathological features that may affect the long-term prognosis after surgical treatment in colorectal carcinomas and explain the different clinical course of patients with similar stages are being investigated. Clearly revealing the histopathological profile of these types of tumors is important in terms of providing patient-based prognostic predictions. In this review article, we discussed the importance of mucin expressions in colorectal carcinomas.

Keywords: Adenocarcinoma, Colorectal, Mucin

Introduction

Basic therapeutic decisions are made by staging the tumor with the AJCC/UICC TNM classification. However, even if diagnosed with the same pathology, patients may benefit from different therapeutic treatments with clinical, other additional immunohistochemical and genetic evaluations besides this staging (Walsh et al., 2013). With the identification of these additional prognostic markers, the contribution of patients to the treatment process cannot be ignored. When the relationship between survival and the main histopathological features that are thought to be associated with poor prognosis, such as the depth of tumor invasion, the status of regional lymph nodes, venous and lymphatic invasion, tumor grade, perineural invasion status, it was found that

perineural invasion, lymph node metastasis, and distant metastasis affect the prognosis (Byrd et al., 2004). It is thought that revealing important histopathological features in colorectal carcinomas will help to predict the prognosis of patients with similar stages and may have important roles in the development of new patient-based therapies. In addition, the relationship between prognosis can be determined by looking at the expression levels by immunohistochemical evaluation.

Mucins are highly glycosylated high molecular weight glycoproteins. They are encoded by different tissue-specific genes and there are 9 different MUC genes (MUC 1, MUC 2, MUC 3, MUC 4, MUC 5AC, MUC5b, MUC6, MUC7, MUC8) (Gendler et al., 1990). It is known that tissue-specific mucin genes and mucin carbohydrate antigens change in colorectal carcinoma and are responsible for the malignant behavior of cancer cells. Although the MUC1 gene is not significantly expressed in normal colon tissue, its expression is increased in colorectal carcinoma and its expression is higher in metastatic tumors. While MUC2 gene expression is decreased

in good-intermediate adenocarcinomas, it is increased in mucinous carcinomas (Byrd et al., 2004; Gendler et al., 1990). MUC3 and MUC4 genes are also significantly expressed in the normal colon, while their expression is increased in adenocarcinoma. While the MUC5AC gene is not expressed in normal colon, they show aberrant expression in colon carcinoma. By determining the structures of antigenic formations formed in these carcinomas, antibodies and vaccines that can be used in diagnosis and treatment have been developed (Velcich et al., 2002). In summary, mucin is a high molecular weight glycoprotein produced by many mucosal epithelial cells. Normally, they protect the cell from microorganisms, toxins and proteolytic enzymes by acting as a barrier between the cell membrane and the external environment on epithelial surfaces (Lugli et al., 2007). In tumoral cells, on the other hand, they provide protection from the immune system by covering the cell surface or covering the surface antigens used in immune recognition. While it is known that MUC1 gene expression is increased in colon adenocarcinomas, *de novo* expression of MUC5AC and MUC6, which are not normally found in the colon, and MUC2 gene expression is increased in mucinous tumors, changes are not clear in adenomas at the beginning of the neoplastic process (Compton, 2007).

Clinical Findings

Colorectal carcinomas are common in Northwest Europe, North America, Australia, New Zealand, and other Anglo-Saxon regions, but are less common in parts of Africa, Asia, and South America (McLeod and Murray, 1999). Worldwide, colorectal carcinomas are generally the third most common in men and the second most common in women. It is a type of cancer and is the second most common cause of death. According to the 2010 cancer statistics of the Ministry of Health, the incidence of colorectal carcinoma in Turkey ranks third among all cancers in men and women (McLeod and Murray, 1999; Lyall et al., 2006).

The etiology of colorectal carcinomas is multifactorial and related to diet, environmental exposure and genetic predisposition. While 50-60% are sporadic in people without risk factors, the incidence is 30-40% in people with a positive family history, colorectal carcinoma or polyp history. It is approximately 5% in hereditary colorectal carcinomas with known genetic transmission pathways (Lyall et al., 2006).

Advanced age is a major risk factor for sporadic can-

cers. Although the incidence increases significantly between the ages of 40-50, it is most common in the age of 55 and above. Although the incidence is close in both sexes, especially rectal cancers are more common in men (9). Low-fiber diets, especially rich in saturated fat and animal foods, increase the incidence. In addition, smoking, alcohol, familial adenomatous polyposis, hereditary nonpolyposis collective cancer syndrome, MUTYH-associated polyposis, juvenile polyposis, Peutz Jeghers syndrome, inflammatory bowel disease are risk factors for the development of colorectal carcinoma. The risk is increased in adults who received abdominal radiation in childhood or in those with a history of radiotherapy for cervical carcinoma or prostate carcinoma (Lyall et al., 2006; Corfield et al., 2001).

Tumors are located in the rectosigmoid region at a rate of 50-60%. Right colon tumors are more common in older age, African people and those with diverticular disease. Colorectal carcinomas may remain asymptomatic for years, and the diagnosis can usually be made after the tumor reaches a certain size (Lyall et al., 2006). Symptoms vary according to the tumor localization, macroscopic features, stage, and the complications that arise. The first and most common finding is a change in bowel habits (constipation-diarrhea, etc.) and is mostly observed in left colon tumors (Corfield et al., 2001).

Iron deficiency anemia is common in tumors located in the right colon, while hematochezia and tenesmus are located in the rectosigmoid region. Abdominal pain can be seen in all regions. Partial obstruction, peritoneal spread or intestinal perforation may cause generalized peritonitis (Byrd et al., 2004).

Stool occult blood test is one of the effective methods in detecting asymptomatic cases. In order to catch the disease at an early stage, endoscopy and colonoscopy examination should be routinely applied to individuals over the age of 40 (Jass and Walsh, 2001). In addition to colonoscopy, radiological methods are helpful in the diagnosis and staging of colorectal carcinomas. Barium enema provides insight into CT colonography, MRI, transrectal US, tumor depth, and regional and distant metastases. However, it should not be forgotten that pathological examination is required for definitive staging in colorectal carcinomas (Lau et al., 2004).

Pathophysiology

In gastrointestinal cancers, mucin glycoproteins are altered in two ways. The first change occurs by incorrect glycosylation. At this stage, incomplete glycosylation and de-O-acetylation of O-acetyl sialic acid cause peripheral false glycosylation (Molaei et al., 2010). The second is that the epitopes in the mucin polypeptide are inappropriately expressed due to sparse and/or insufficient glycosylation, alteration of transcription or dysregulation of the mucin gene (Jass and Walsh, 2001; Lau et al., 2004).

Aberrant glycosylation: While the change in the expression of carbohydrates (CH) in the core region is due to the completed synthesis, the change in the expression of carbohydrates in the peripheral regions occurs due to the elongation or change of the existing structure (Molaei et al., 2010). Thus, the CH side chains of the mucin glycoprotein in colon cancer cells acquire the antigenic epitope structure observed in cancers either in the core region or in the peripheral and backbone structure. As a result of aberrant glycosylation of CH in the core region, CH with shorter chain antigenic structures such as T, Tn, Sialy Tn and Sialy T emerge (Biemer-Hüttmann et al., 2000). Studies have found that the expression of these antigens is increased in adenomatous polyps, and their expression is higher in colon carcinomas (Molaei et al., 2010; Biemer-Hüttmann et al., 2000).

After the change of peripheral or backbone Squamous Cell Carcinoma antigenic CH chains such as Le and Sialy Le emerge (Lan et al., 1990). While these antigens are not found in normal colonic mucosa, their expression is increased in adenomatous polyps and colon carcinomas. Various studies have shown that Sialyl Le antigen is overexpressed in metastatic colon cancers and can be used as a prognostic marker. Decreased O-acetylation in cancer cells increases the metastatic potential (Biemer-Hüttmann et al., 2000; Lan et al., 1990). It has been stated that the high expression of Sialyl Le is a factor that may indicate recurrence. In addition, both Sialy Tn and extended Sialyl Le antigens can be used as prognostic markers. Primary tumors expressing these antigens have a poor prognosis (Ligtenberg et al., 1990).

In summary, repeated sequences in the central tandem repeat region of normal mucin glycoprotein are extensively glycosylated, many CH side chains are present, and each chain is long. With malignant transformation, the tandem repeat regions are less frequently glycosylated and the CH chains are shorter and/or located in the outer region. These chan-

ges occur due to altered CH metabolism or change in glycosyl transferase or O-acetylation of sialic acid. Thus, the modified sugar structure or the inner sugar structure or protein core epitopes emerge and become visible (Ho et al., 1993). These antigenic epitopes are recognized by cytotoxic T lymphocytes and produce antibodies against them.

While the MUC1 antibody is strongly expressed in mucus-forming cells in the gastric surface epithelium and neck epithelium, they also show perinuclear expression in crypt cells and submucous glands in the duodenum, jejunum or ileum (Regimbald et al., 1996). MUC1 expression is found in the apical membranes of ductal glandular tissues such as the gallbladder, esophagus, normal breast tissue, prostate, endometrium, and endocervix. They are found in the apical membranes of the pancreatic ducts and lobules. MUC1 is present at very low levels in normal colon tissue. In addition to colon cancer tissue, MUC1 can be detected at very low levels in normal tissue. MUC1 expression in normal tissue adjacent to colon cancer tissue is the same as in normal colon. MUC1 stains cytoplasmic in normal colonic crypts, apical membrane and/or diffuse cytoplasmic staining (Regimbald et al., 1996; Kaira et al., 2012).

MUC1 expression is increased in cancerous tissues compared to lung cancer, breast cancer, normal bronchial tissue and breast tissue. MUC1-related epitopes have been demonstrated in 80-90% of cases in gastric carcinomas. Strong MUC1 expression has been shown in pancreatic, prostate cancer and esophageal cancers such as breast and lung (Kaira et al., 2012). While MUC1 is expressed at a very low level in normal colon, it is expressed at a rate of 70-85% in colon cancer. Immunoreactivity is localized to the cytoplasm, cell membrane, and luminal contents of malignant glands. Despite the appearance and increase of MUC1-related epitopes in colon cancer, MUC1 mRNA levels are the same or lower than in normal colon. This suggests that MUC1 immunoreactivity occurs after the change occurring at the post-transcriptional level (Nagai et al., 2006).

MUC1 expression has been shown to be significantly increased in severe dysplastic adenomas and tubular differentiated tumors. MUC1 expression was also found to be high in metastatic liver tissue. It shows a significant difference in expression in the primary tumor compared to normal tissue. Considering that it is not found in normal colon tissue, it can be stated that MUC1 expression can be used as a marker showing malignant transformation (Jarrard et al., 1998).

It has been shown that tumor-specific cytotoxic T lymphocytes are activated in the body against MUC1 epitopes. In another study, there was no correlation between MUC1 expression and tumor stage, tumor localization and differentiation in patients with Dukes B and D tumors, while a positive correlation was found between the percentage of tumor cells expressing MUC1 and the number of cytotoxic T lymphocytes. In the light of these studies, immunotherapy and vaccine against MUC1 have been developed (Sierzega et al., 2016).

Expression of the MUC2 gene in normal tissues was limited in the intestinal epithelium (Jarrard et al. 1998; Sierzega et al. 2016). Both in immunohistochemical studies and in situ hybridization studies MUC2 apomucin showed that it is located supranuclear and perinuclear in small intestine, normal colon and colon cancerous tissues, goblet cells. They can be found in goblet cells in all regions of villi and crypts. They are stored in granules within MUC2 goblet cells, are released after the stimulation mechanisms and act as lubrication and protection (Ookawa et al., 2002).

Other tissues in the gastrointestinal tract; stomach, esophagus and gallbladder are not at a level to produce a significant amount of MUC2. While it is rarely found in the tracheobronchial epithelium, MUC2 mRNA and apomucin expression are not found in the lung, breast, pancreas, prostate, seminal vesicle, and endocervix (Gum et al., 1994). While MUC2 mRNA levels were found to be high in normal colon tissue, they were found to be normal in small intestine tissue. The expression of the MUC2 gene in transitional mucosa is the same as in normal colon tissue. The expression of the MUC2 gene in adenomas depends on the frequency and intensity of expression of cancer-dependent glycoprotein antigens in adenomatous polyps, the increased risk of malignancy of the polyp, and the size of the polyp (Axelsson et al., 1998).

In a study by Blank et al. MUC2 protein epitope was found to be strongly positive in normal colon tissue in 21%, while it was moderately expressed in 65% (Godl et al., 2002). In the same study, it was found 40% strongly positive in villous adenomas and 48% strongly positive in tubular adenomas. In a study by Godl et al. (2002), MUC2 overexpression was observed in 73% of patients with rectosigmoidal villous adenoma, and it was stated that this expression rate did not change with the degree of dysplasia. In a study by Van et al. (2006), it was observed that MUC2 expression decreased as the dysplasia type progressed towards severe dysplasia in both flat and polypoid type adenomas. While MUC2 expression is obser-

ved at a rate of 92% in low-grade dysplasia, this rate decreases to 60% in high-grade dysplasia (Van et al., 2006). Decreased MUC2 expression in high dysplasia may be attributed to decreased mucin formation as dysplasia increases. In some studies, it has been shown that the expression intensity of MUC2 apomucin in colon cancer is increased in well and moderately-well differentiated colon cancers compared to normal colon. While MUC2 mRNA levels were increased in mucinous cancers compared to normal colon, it was observed that mRNA levels decreased in well- and moderately-well-differentiated adenocarcinomas (Hanski et al., 1997).

In the study by Hanski et al. (1997), it was observed that the expression of anti-MUC2 antibody (CCP58) increased in mucinous carcinomas, while the expression was normal or decreased in denova and adenocarcinoma sequence (ACS) (Vincent et al., 2007). While MUC2 antibody was detected in normal colon tissue at a rate of 86%, it was reported that they showed 59% expression in ACS and denova carcinomas, and the expression was weaker in denova carcinomas. In mucinous carcinoma, on the other hand, MUC2 shows strong overexpression at a rate of 74%. MUC2 expression is independent of the degree and stage of differentiation (Mesquita et al., 2003).

Both MUC2 apomucin and MUC2 mRNA expression are increased in mucinous carcinomas. More apomucin, shorter chain and less carbohydrate chain apomucin is synthesized (Yamamoto et al., 2003). The MUC2 epitope becomes more visible as a result of increased expression of both MUC2 apomucin and MUC2 mRNA, insufficient glycosylation and/or abnormal mucin formation. CCP58 antibody is intracellularly located and does not stain secretory mucin and mucin pools. Because secreted mucin is mature, it is highly glycosylated and is not stained by CCP58 and other antibodies. However, in some studies, it was observed that luminal and interstitial mucin showed MUC2 expression (Weiss et al., 1996).

The MUC5AC gene encodes for secretory mucin, and is strongly expressed in gastric superficial and neck mucus cells. They show expression in the glandular epithelium of the respiratory system, in the gallbladder epithelium in the endocervix. The MUC5AC gene is not expressed in normal colon tissue but it has been found to be expressed in nonneoplastic mucosa close to cancer tissue. In some studies, it has been shown that there is an expression of 5-20% in the transitional mucosa (Walsh et al., 2013). In some studies, the expression of distant normal colon tissue in cancer tissue, albeit very little, has been explained by

the 'precancerous field effect'. In another study, expression was observed in the normal colon at a rate of 14%. It has been shown that MUC5AC and MUC6 protein and mRNA levels are found in very little or no amount in normal colon tissue, while denovo expressions are found in colonic polyps (Bu et al., 2010).

In a study by Buisine et al., MUC5AC was shown to be aberrantly expressed in all cases with rectosigmoidal villous adenoma. While MUC5AC expression was found to be stronger in 91% of patients with low-grade dysplasia, weak expression was observed in 9% (Jia et al., 2010). This rate decreased in patients with high grade dysplasia. (25% strong 75% weak). While staining was detected in 33% of cases with high grade dysplasia positive invasive adenocarcinoma, no expression was observed in tissues with adenocarcinoma. In a study by Bartman et al., they looked at the expression of MUC5AC and MUC6 in normal colon mucosa hyperplastic polyps and adenomatous polyps, and rarely low-intensity staining was observed in normal colon and hyperplastic polyps (Kesari et al., 2015). High staining was evident in cases of moderate dysplasia with moderate villous structures in large adenomas. Similarly, in the same study, MUC5AC, MUC6 mRNA levels were examined with the slot blot analysis technique, and high expression was found in moderate dysplasia cases with medium-sized moderate villous structures (Terada et al., 2013).

While MUC1, MUC3 have been shown to be expressed in both normal and hyperplastic colonic cells, MUC5AC and MUC6 are not detected in normal colon tissue and show denovo aberrant expression in adenomatous polyps, although it does not show malignant transformation in the colon but can be used as specific markers (Bu et al., 2010). It has been reported that the MUC5AC gene is expressed in pancreatic adenocarcinomas and mucinous carcinomas. In one study, it was observed that it was expressed at a rate of 93%. There are not many studies indicating the percentage of expression in colon carcinoma. Only Bara et al stated that MUC5AC expression was 12-29% (Losi et al., 2004).

Glycoproteins play a role in many steps of metastasis, cell growth, invasion, relationships between different tumor cells, in the immune system, and in the production of metastatic cells into the endothelium, platelets, and extracellular matrix (Kocer et al., 2002). It has been shown that the prognosis is worse in tumors with more mucin formation. It was also observed that the metastatic potential and liver colonization increased with the increase in mucin formation in human colon cancer cells. It has been shown that the binding

of laminin type 4 collagen and fibronectin to basement membrane proteins is higher in human colon cancer cells that produce high mucin than in human colon cancer cells that form low mucin (Kocer et al., 2002; Bartman et al., 1999). This correlates directly with the mucin-forming capacity of colon cancer cells to invade the basement membrane. When O-glycosylation was inhibited with benzyl alpha N acetyl galactosamine, decreased cell adhesion to matrix proteins was observed. Type 4 collagenase activity promotes basement membrane degradation and movement in the extracellular matrix. Type 4 collagenase activity is higher in colon cancer cells with high mucin secretion (Boonla et al., 2005). Sialated glycoproteins have been observed to be more common in metastatic tumors than non-sialated antigens. Sialyl T, Sialyl Tn, Sialyl Le antigens and sialic acid levels in metastatic cells are twice as high as in nonmetastatic cells. High amounts of sialyl Tn antigens play a role in binding to the extracellular matrix and Le antigens in binding to selectins (Kocer et al., 2006).

Conclusion

In summary, mucin increases adhesion and attachment to normal colonic cells, while stimulating collagenase activity, allowing tumor cells to invade the basement membrane. Mucins can hide sialic acid fragments and antigenic epitopes from the immune system, thus mucin-dependent TN antigens prevent inhibition of leukocytes and activation of natural killer cells (Betge et al., 2016).

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