Mucin and Colorectal Cancer

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Mucin is a high molecular weight glycoprotein that is synthesized, stored and secreted by the epithelial mucosal cells, especially the goblet cells [1]. The mucin molecule is highly glycosylated with long sugar side chains attached to the protein backbone (especially the amino acids serine and threonine) through oxygen bonds. These bonds prevent the breakdown of mucin by proteases, and provide density and viscosity. Thus, mucin can efficiently fulfil its role as the main constituent of the mucus-protecting layer in the gastrointestinal tract. The tandem repeat peptide, rich with serine and threonine and containing the dense sugar side chains, is typical of the different mucin species.

Mucin proteins are derived from multiple different genes, termed MUC genes. These include MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6 and MUC7 [2–11]. MUC1 gene is located on chromosome 1 and encodes for 20 amino acid tandem repeats. This is a membrane-associated mucin and is expressed on most of the epithelial cells. All the other mucins are secretory mucins. MUC2 and MUC3 are expressed in the colon, while MUC5 and MUC6 are typical for the stomach [12].

Several lines of evidence point towards a biological role of mucin in colorectal cancer [13–40]. These include: observations on mucinous colorectal cancer, *in vitro* and *in vivo* experiments, alterations of mucin structure in polyps and cancer, and a new approach in cancer vaccination.

Observations on mucinous colorectal cancer [13–29]

Mucinous colorectal cancer and the rare signet ring cell colorectal cancer have a higher Dukes stage at diagnosis and a worse prognosis [13–16]. The mucinous cancer has a higher penetration rate, increased lymph node involvement, less protective lymphocyte infiltration in the tumor margins, higher metastatic potential, and lower 5 year survival rate [13]. Recent papers have confirmed these observations and characterized several additional factors typical of mucinous colorectal cancer patients [17–26]. A worse prognosis and more cases of treatment failure, especially for patients with Dukes B cancer, have been described [17]. Suma and Nirmala [18] found a predilection for mucinous colorectal cancer at a younger age and a higher incidence in the proximal colon. They also found that the tumor's aggressive behavior and poor prognosis correlated with the percentage of mucinous component, independent of the histological grade [18]. These observations may be important when making treatment decisions since colorectal carcinoma showing mucinous component in the pre-operative biopsy is significantly more likely to reveal a high mucin content and to be at an advanced stage at resection [19]. Yamamoto et al. [20] demonstrated a higher invasion rate of mucinous cancer to the adjacent viscera and greater lymph node involvement beyond the pericolic region than non-mucinous cancer. In series from Louisiana [21], Genoa [22], Taipei [23] and Tokyo [24], mucinous tumors presented at a statistically significant more advanced stage. The two types of tumors (mucinous cancer and non-mucinous adenocarcinoma) differed also with respect to somatic genetic mutations [25,26]. Compared with non-mucinous carcinoma, mucinous cancer had a higher k-ras mutation rate, but less p53 expression.

In vitro experiments [27,28]

In vitro (cell line) experiments demonstrated increased proliferation, clonigenicity and invasiveness in cell lines that synthesized and secreted large amounts of mucin [27]. When cell lines (LS174T, CACO2, T84 and HT29) were compared on the basis of the same cell number, the higher mucin production rate was correlated with higher colony-forming efficiency.

Low mucin-producing cell lines (LS174T, LM 12) are significantly weaker in basement membrane adhesion and invasion than high mucin lines (HM 7, HM 3, LS LiM 6) [28]. Adhesion of low mucin-producing cell lines to various matrix proteins and secretion of type IV collagenase is potentiated by the addition of purified human colon cancer mucin in a dose-dependent manner.

In vivo experiments [27,29–31]

In vivo studies in experimental animals demonstrated increased tumorigenicity and metastatic potential of tumors derived from cell lines that produced large amounts of mucin [27–29]. Cell line-increased tumorigenicity was correlated with high mucin synthesis [29]. Three lines of evidence indicate that the amount of mucin produced is functionally important to the metastatic capacity of human colon cancer cells. First, highly metastatic colon cancer cell lines have more mucin than do cells with low metastatic ability. Highly metastatic LS LIM 6 tumors produce a greater amount of metabolically labeled

intracellular mucin and a four to fivefold increase in secretion of mucin when compared with the poorly metastatic parental line LS174T. There was also an elevation in MUC2 mRNA and mucin carbohydrate antigens. Second, high mucin-producing cell lines are more metastatic than cells that produce a smaller amount of mucin. The ability of variants selected for high (HM 7) or low (LM 12) mucin synthesis to metastasize and to colonize the liver is proportional to their production of mucin. Third, inhibition of mucin glycosylation by arylglycoside reduced liver colonization [30,31]. The inhibitor of mucin glycosylation can be used to inhibit the expression of peripheral carbohydrate antigens on mucin-like glycoproteins as a tool to further define the role of mucins in the biological behavior of colon cancer cells.

Alterations of mucin structure in polyps and cancer [32–38]

Certain alterations of mucin in the cytoplasm and secretions of polyps have been observed. These include: a) aberrant glycosylation leading to reduced number and length of carbohydrate side chains on mucin core protein, b) enhanced core peptide immunoreactivity, c) deletion of normally expressed antigens, d) reappearance of normal antigens, e) *de novo* appearance of novel antigens, and f) expression of blood group-incompatible antigens.

The differences from normal mucin have been described both at the gene level and post-translational, namely incomplete synthesis or incomplete glycosylation exposes core epitopes and several new antigens. An altered pattern of expression of mucin genes may be found in the cancer tissue. Since fewer oligosaccharides are attached to the backbone of the mucin in cancer, specific cancer antigens are exposed that may have a role in cancer diagnosis and immunological response. Core antigens as T, Tn and Sialyl-Tn, and peripheral antigens as Lewis blood groups may be present [32,33]. The synthesis of MUC 2, a regular goblet cell mucin, is suppressed in colorectal cancer and metastasis [34], and its distribution within the cell changes [35]. MUC2 expression was found to increase in the cytoplasm and Golgi apparatus and decrease in the goblet cell vesicle in adenomas compared to normal mucosa. MUC 5AC and MUC6, regular gastric mucins that make the unstirred layer of the protecting gastric mucus, were found in a high proportion of villous and tubovillous adenomas, but not in normal colonic mucosal biopsies [36].

Changes in mucin pattern may affect the behavior of the disease. When MUC1 was expressed at the deepest tumor invasive portion, lymphatic and venous invasion was more pronounced as found in lymph nodes and liver metastasis [37].

Glycosylation of mucin backbone is controlled by glycosyltransferases [1]. These may also change in cancer tissue. Up-regulation of UDP-galactose-N-acetyl-galactose-amine I and II was found in cancer versus normal colonic mucosa [38]. Higher levels were found in Dukes C cancer compared with Dukes A and B.

A new approach to cancer vaccination [39,40]

Novel mucin epitopes are expressed by tumor cells owing to aberrant glycosylation, shorter sugar side chains and exposure of peptide antigens. Specific T cells for native polypeptide core tandem repeat can be expanded *in vitro*, and humoral response may be generated with B cells recognizing the mucin tandem repeats. One example is the detection of circulating anti-MUC1 antibodies in patients with colorectal cancer. The way for cancer immunization research is now open, and already considerable progress has been made. For example, a vaccine has been formed by transecting MUC1 gene into Epstein-Barr virus-immortalized B cells. The latter act as antigen-presenting cells priming cytotoxic T cells. Another example is the development of 105-amino acid MUC1 peptide, which is admixed with Bacille Calmette-Guerin and injected into patients with advanced colorectal cancer [39]. Results showed a two to fourfold increase in CD8 lymphocyte count in 7 of 22 patients. The third example is immunization with Sialyl-Tn, which led to a detectable immunoglobulin G response [40]. It was found that the higher the IgG titers, the longer the patient's survival time.

Conclusion

Mucin synthesis and secretion by colorectal cancer has a role in the biological behavior of the disease, as demonstrated *in vitro* and *in vivo*. Expanding our knowledge by colorectal cancer mucin research may help us better understand the natural history of this devastating disease and thereby contribute to the development of new treatment strategies.

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