Biomolecular Markers as Determinants of Patients Selection for Adjuvant Chemotherapy of Sporadic Colorectal Cancers

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ABSTRACT

Colorectal cancer (CRC) is a disease classified and based on genetic alteration resulting from interaction of environmental factors, individual cancer susceptibility and accumulated somatic changes of the colorectal epithelium. Advanced knowledge in genetics and epigenetics of colorectal cancer develops a hypothesis that various clinical manifestations of colorectal cancer are caused by different carcinogenesis pathways. Different carcinogenesis pathways and types of colorectal cancer appear to bring effects on different response against chemotherapy and prognosis.

Chemotherapy is mainly provided for patients with stage III CRC which are also the largest proportion of CRC patients in Indonesia. However, it is also provided for some patients with high risk stage II CRC. Classically, clinical factors have been generally accepted as prognostic factors including depth of tumor invasion, regional nodal metastasis, vascular invasion, poor differentiation, and serologic tumor marker such as carcinoembryonic antigen (CEA). However, clinical and histopathological factors themselves do not provide accurate prediction for colorectal cancer prognosis and treatment. A biomolecular marker is necessary to provide such prediction.

Numerous studies have been conducted to evaluate the molecular biological markers in order to determine either the possibility of successful treatment for colorectal cancer (predictive factor) or life-expectancy (prognostic factor). Results of several studies demonstrate different status of some molecular markers to determine successful treatment between stage II and stage III colorectal cancer. Certainly, such finding should be followed up but it shall be accepted that there will be a shift of paradigm of CRC treatment. Therefore, the success of colorectal cancer, excluding the patient’s socioeconomic factors and the surgeon’s skill, will depend extremely on molecular parameter and not only the stage.

Key words: colorectal cancer, chemotherapy, biomolecular markers, gene mutation.

INTRODUCTION

Colorectal cancer (CRC) is a disease classified based on genetic alteration resulting from interaction of environmental factors, individual cancer susceptibility and accumulated somatic changes of the colorectal epithelium. Such accumulated genetic alteration of normal colorectal epithelial cells then is inherited to their progeny which leads to deterioration from benign adenoma into invasive carcinoma.

The accumulated genetic alteration occurs at least through two different mechanisms. The first is chromosomal instability (CIN), which is correlated to the loss of heterozygosity (LoH) on various tumor suppressor locus, particularly on chromosome 18q. CIN occurs in approximately 80-85% sporadic CRC. The second mechanism is through microsatellite instability (MSI) pathway. In this pathway, there is lack of DNA proteins for mismatch repair (MMR) system, two of the most important proteins are MSH2 and MLH1.

Advanced knowledge in genetics and epigenetics of colorectal cancer develops a hypothesis that various clinical manifestations of colorectal cancer are caused by different carcinogenesis pathways. Therefore, it is assumed that there are several types of colorectal cancers. Such different pathways are demonstrated by different genomic instability and mutation of certain genes such as p53, APC, and K-RAS. The most frequent combined mutation is mutation of p53 and APC; while the mutation of p53 and K-RAS are less frequent. Mutation of such genes is also associated with certain aberrant chromosom which also represents certain carcinogenesis pathway.

Different carcinogenesis pathways and types of colorectal cancer appear to bring effects on different response against chemotherapy and prognosis. Chemotherapy is an important modality in CRC treatment, either as adjuvant or primary treatment.
Chemotherapy is mainly provided for patients with stage III CRC which are also the largest proportion of CRC patients in Indonesia. However, it is also provided for some patients with high risk stage II CRC, i.e. patients who present with tumor of T4N0M0, 3rd or 4th histopathological grade, peritumoral lymph and vascular invasion, obstruction or perforation, R1 resection and patient with fewer than 12 resected regional lymph nodes.  

Classically, clinical factors have been generally accepted as prognostic factors including depth of tumor invasion, regional nodal metastasis, vascular invasion, poor differentiation, and serologic tumor marker such as carcinoembryonic antigen (CEA). However, clinical and histopathological factors themselves do not provide accurate prediction for colorectal cancer prognosis and treatment. A biomolecular marker is necessary to provide such prediction. The present article will describe state-of-the art on the role of biomolecular marker as predictive and prognostic factors for colorectal cancer.

**MICROSATELLITE INSTABILITY**

Approximately 15-20% sporadic CRC demonstrates high grade of MSI or known as high MSI (MSI-H). Most of sporadic CRC have MSI-H that occurs due to epigenetic events, i.e. hypermethylation of CpG Island on hMLHI gene promoter region. A present, immunohistochemistry for MSH2 and MLH1 is regarded as adequate examination for detecting microsatellite instability.

A study of 97 sporadic CRC patients in Indonesia indicated that there is no different expression of MSH2 and MLH1 protein between patients aged 40 years or less and aged 60 years or more. Negative expression of MSH2 was found in 43.5% cases; while negative expression of MLH1 was found in 83.5% cases. The negative expression was observed in 15/15 (100%) cases of proximal colon tumor and 66/82 (80.5%) cases of distal colon tumor (p = 0.061). A study on gene expression profiling demonstrated that distal CRC with MSI has different expression profile from proximal cancer, which leads to an impression that distal cancer with MSI is a different subgroup of sporadic MSI cancer.

It shall be anticipated that in a short time, microsatellite instability testing will become part of routine evaluation for colorectal cancer revealing the prognostic factors.

**K-RAS GENE MUTATION**


K-ras mutation is associated with worse prognosis. However, there is no convincing evidence that K-ras mutation could be an independent prognostic factor of colorectal cancer. A large-scale study involving 3,439 CRC patients found that of the 12 possible mutations on codons 12 and 13, only the glycine to valine mutation on codon 12 (8.6%) had statistically significant to the patient’s outcome. Similar result is also obtained by other study, but data of large randomized retrospective studies failed to demonstrate a significant and consistent effect of K-ras mutation on prognosis of CRC patients.

The K-ras mutation status appears to have a role in treatment response using anti-epidermal growth factor receptor (EGFR) monoclonal antibody. A phase III randomized clinical trial, i.e. the CRYSTAL trial evaluated the regimen of cetuximab and irinotecan-containing infusional fluoro-uracil (FU), bolus and infusional FU-leucovorin-irinotecan as the first-line treatment of patients with metastatic CRC who had EGFR expression. The study found statistically significant improvement in terms of overall response rate and median progression-free survival (PFS) in the groups receiving cetuximab regimen. In a subset of patients
whose K-ras mutation status could be analyzed, the benefit of cetuximab was limited in patients without K-ras mutation.34

Other study, the Phase II OPUS trial evaluated FOLFOX-cetuximab regimen at the first-line treatment. Patients with wild-type K-ras enjoyed benefit of cetuximab treatment as adjuvant therapy compared to patients who only had FOLFOX of response rate 61% vs 37%; p = 0.01 and PFS 7.7 vs 7.2 months; p = 0.02

The randomized trial of Cancer and Leukemia Group B (CALBG) 89803 involved 1264 patients with phase III CRC who had weekly bolus of FU/leucovorin or bolus of irinotecan, FU and leucovorin (IFL) following surgery. The primary end point was overall survival (OS); while the secondary end point was disease-free survival (DFS). MMR defect (MMR-D) was indicated by missing protein expression of MLH1 and MSH2 in immunohistochemistry staining; while the MSI-H genotypes were detected by using mono- and dinucleotide markers panel. Patients who had received IFL with MMR-D/MSI-H tumor demonstrated improvement of 5-year DFS compared to the tumors with intact mismatch repair. Such correlation was not found in the patient group treated by FU/LV. MMR dysfunction may predict better outcome for patients treated with IFL regimen compared to the FU/LV.35

**BRAF MUTATION**

The frequency of BRAF mutation 735 patients aged <60 years was 7%, which was very significantly correlated to advances stage (III-IV), proximal colon tumor sites and high histopathological grade. The frequency of such mutation was also tends to be higher in the very young aged patients (<40years).36

BRAF mutation and DNA methylation contributes on sporadic colorectal cancer with MSI-H as a particular carcinogenesis pathway, which is now known as the methylator pathway.37 Recent study on sporadic CRC in patients aged <60 years found a case with BRAF mutation which also had CpG island methylator phenotype (CIMP).38

BRAF gene is assumed to bring advantage as the K-ras gene in predicting the treatment reponse to EGFR inhibitor. After 6-month tretament, patients who had BRAF mutation compared to patients with normal BRAF showed greater number of patients with aggravating cancer (82 vs. 59%) and have lower overall survival (30 vs 85%). Other studies demonstrate that treatment by using BRAF inhibitor, sorafenib may restore sensitivity against EGFR inhibitor of the colorectal cancer cells that have experienced BRAF mutation.38

**P53 MUTATION**

Product gene of p53 on protein p53 has a function in DNA reparation mechanism. Normal p53 protein (wild-type) causes cell cycle arrest when DNA damage occurs, so the DNA can be corrected if there is minor damage or the cell will experience apoptosis when the damage is severe and uncorrectable. A loss of p53 normal function may induce genomic instability due to genetic error which will be replicated and causing loss of heterozygosity (LoH).

Mutation of p53 gene is an important step on transition of advanced adenoma into adenocarcinoma. Approximately 50% of lesions with high degree of colon dysplasia and 75% lesions of colon cancer demonstrate mutation of p53 gene.43

**18qLOH/DCC**

LOH on chromosome 18q has been associated with poorer OS outcome in patients with high-risk stage II / stage III colorectal cancer who had received 5-FU-based chemotherapy.

In a study by Cancer and Leukemia Group B (CALGB) protocol 9581, 1783 patients with stage II received 500 mg loading dose of monoclonal antibody 17-1A following resection plus four infusion of 100 mg every 28 days on observation. The primary end point was OS and DFS was considered as the secondary end point. The status of 18qLOH was evaluated. Patients with positive 18qLOH tumor significantly had reduced DFS and OS. Five-year DFS in patients with positive 18qLOH was lower compared to negative
18qLOH (0.85 vs. 0.98; HR = 0.25, 95%CI 0.07-0.83; log rank p = 0.01). LOH on 18q chromosome is a prognostic factor for DFS and OS in patients with low-risk stage II colorectal cancer who have not received adjuvant chemotherapy.46

SMAD4 GENE MUTATION

One of the most common cytogenetic abnormalities in colorectal tumor is the loss of genetic material in chromosome 18q.21 The identity of gene targeted by deletion in 18q has remained elusive. Some genes have been proposed including SMAD2, DCC (deleted in colorectal carcinoma), and SMAD4.6,47,48 Somatic mutation of SMAD4 is presumed to have a role in carcinogenesis of human colorectal tumors.49,50 The median OS of patients with low SMAD4 levels was far shorter compared to the high SMAD4 levels (1.7 years vs >9 years, p = 0.025) indicating that low SMAD4 protein level is a poor prognosis for patients with Duke C colorectal cancer.51

THE EXAMINATION OF MULTIPLE MOLECULAR MARKERS

Research by using some molecular markers all at once appears to potentially provide better information in determining treatment response and prognosis. For example, the National Surgical Adjuvant Breast and Bowel Project (NSABP) developed and conducted validation against the first multigene assay to predict the risk of recurrence in patients with stage II colorectal cancer. The assay is called Oncotype DX (Genomic Health, Redwood City, CA) and it produces recurrence score that can be utilized by doctors to determine the possibility of recurrence in patients. End points in NSABP include recurrence-free interval (RFI), disease-free survival (DFS), and overall survival (OS), which were studied in 1851 patients with stage II colorectal cancer. Multivariate analysis was conducted in terms of stage, histopathological grading, lymph nodes, MSI status, which found 18 genes, i.e. 7 prognostic genes, 6 predictive genes and 5 reference genes as well as the algorithm of prognostic recurrence score (RS) and predictive treatment score (TS). The results indicated that RS could predict DFS and OS. On multivariate analysis, RS may become an independent prognostic indicator of MMR, T stage, the number of examined lymph nodes, histopathological degree and lymphvascular invasion. Other independent prognostic factors were MMR and T4 deficiency. Treatment score has not been validated yet.52

Evaluation of various biomolecular marker has also been conducted at the level of protein expression by using paraffin block specimen and immunohistochemistry technique. One of recent clinical trials is the prospective adjuvant colon cancer trial 3 (PETACC-3) which attempts to develop biomolecular marker in the context of multicenter clinical trial.53

PETACC-3 is a randomized phase III clinical trial assessing the role of irinotecan on fluorourasil/leucovorin regimens as adjuvant therapy in 3,278 patients with stage II and III colorectal cancer.54 In some cases (1,563 patients), the specimens obtained following the tumor resection were evaluated further to the molecular level, i.e. expression of p53, thymidylate synthetase (TS), hTERT, SMAD, K-ras and BRAF mutation, microsatellite instability (MSI) and 18qLOH. The result of the study was surprising since there was a significant difference regarding prognostic value of various molecular marker between stage II and stage III, i.e. the size of T and MSI as the independent predictor of stage II and the N, T, SMAD4 and p53 status as the independent predictors of stage III. In conclusion, molecular markers for colorectal cancer have prognostic value which is stage-specific. Such analysis makes an impression that stage II and stage III colorectal cancer tend to be different disease rather than the same disease of different stage.55,56

CONCLUSION

Numerous studies have been conducted to evaluate the molecular biological markers in order to determine either the possibility of successful treatment for colorectal cancer (predictive factor) or life-expectancy (prognostic factor). Results of several studies demonstrate different status of some molecular markers to determine successful treatment between stage II and stage III colorectal cancer. Moreover, based on data input, the result was quite surprising, i.e. some reports of the World Congress of Gastrointestinal Cancer in Barcelona in 2009, for example, provided an impression that stage II and stage III colorectal cancer are different diseases. Certainly, such findings should be followed up but it shall be accepted that there will be a shift in paradigm of CRC treatment.

Therefore, the success of colorectal cancer, excluding the patient’s socioeconomic factors and the surgeon’s skill, will depend extremely on molecular parameters and not only on the stage.
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