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REVIEW ARTICLE

Prognostic and Molecular Factors in Stage II Colorectal Cancer

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Over the last 20 years, adjuvant chemotherapy has been administered after surgical resection of tumors for colorectal cancer (CRC) patients with stage III disease to reduce the risk of recurrence of cancer. However, it is controversial as to whether all stage II CRC patients, or at least stage II CRC patients with additional risk factors, should receive adjuvant chemotherapy. Adjuvant chemotherapy in stage II CRC patients may be considered for patients in high-risk groups. It is a high priority to define prognostic factors for these stage II CRC patients to identify high-risk patients at risk of tumor metastases or recurrence and referral of stage II CRC patients for individual assessment. Recent guidelines advocate the consideration of clinicopathological factors such as free bowel perforation or obstruction, lymphatic and vascular invasion, poorly differentiated tumors, fewer than 12 lymph nodes examined, tumors with adjacent organ involvement, and indeterminate or positive margins as strong predictors of a poor prognosis in stage II CRC. Furthermore, with recent advances in basic research attempting to elucidate the underlying molecular mechanisms of carcinogenesis, a variety of candidate genes with potential value for the early detection of cancer have been discovered. Molecular factors such as microsatellite stability and loss of heterozygosity of 18q have been used to identify groups of patients with stage II CRC who have much worse prognoses and may benefit from administration of chemotherapy. Accumulated reports have described the detection of circulating tumor cell-related molecular markers in the peripheral blood of CRC patients, which has important prognostic and therapeutic implications. Consequently, therapeutic decision-making models are likely to be further refined by the inclusion of such molecular markers.

Key Words: molecular factors; prognostic factors; stage II colorectal cancer

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Introduction

Colorectal cancer (CRC) is a significant problem worldwide, and while it accounts for 10% of all cancers, it is the third leading cause of cancer deaths in the Western world.[1] CRC is one of the most common malignancies in Taiwan, and is also the third major cause of cancer deaths in Taiwan. More than 10,000 new cases were diagnosed, and over 4100 patients died from this disease in 2007. Consequently, the incidence of CRC has gradually approached Western levels in recent decades. The most important prognostic indicator for survival in CRC is the tumor stage. Currently, TNM classification from the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) staging systems for CRC, which is determined by depth penetration through the bowel wall, the number of lymph nodes, and the presence of distant metastasis involved, provides more detailed information than other staging systems.[2]

Adjuvant chemotherapy with 5-fluorouracil (5-FU)/leucovorin modulated by folinic acid combined with oxaliplatin significantly improves 5-year disease-free survival and 6-year overall survival in stage III colon cancer patients, and it should be considered after surgery for patients with stage III disease.[3] However, the use of adjuvant chemotherapy for stage II patients remains controversial. Patients with stage II colon cancer constitute a particularly heterogenous population. Routine chemotherapy administration may be inappropriate and costly for all stage II patients;[4] therefore, it is not recommended that patients with stage II CRC undergo routine adjuvant chemotherapy, compared with patients with positive lymph nodes (AJCC stage III).[5]

However, approximately 25–30% of CRC patients with stage II disease are at high risk for postoperative relapse. Indeed, the clinical outcome of patients with high-risk stage II disease is similar to that of patients with stage III disease.[6] Therefore, it is important to identify high-risk stage II CRC patients for whom adjuvant chemotherapy may yield potential benefits.

In recent guidelines, factors such as free bowel perforation or occlusion, lymphatic and vascular invasion, depth of tumor invasion and adjacent organ involvement, detection of occult neoplastic cells in lymph nodes, and extramural venous invasion and peritoneal involvement have been considered when assessing the likely benefit-risk ratio. More importantly, molecular markers such as loss of heterozygosity of 18q or presence of microsatellite instability (MSI), allelic imbalance, and levels of thymidylate synthase have facilitated the identification of subgroups of patients with stage II CRC who should (or should not) be treated.[7] Therefore, molecular biomarkers might be valuable for identification of high-risk stage II CRC patients.

It is well known that tumor cell dissemination and formation of metastases are the result of multiple steps involving patient- and tumor-related factors. Possible mechanisms for the persistence of disseminated tumor cells in the circulating venous or lymphatic streams may be an immune escape mechanism or a higher malignant potential of this specific clone. Disseminated tumor cells in blood and bone marrow have been shown to be valid markers in patients with CRC.[8] However, none of these markers are currently used in clinical practice for making decisions on whether a patient with stage II CRC should receive adjuvant chemotherapy. Recently, detection of circulating tumor cells in blood samples of patients with stage II CRC has identified patients with poor outcomes.[8] However, this finding needs to be confirmed by further large studies to evaluate whether these factors are appropriate as prognostic markers in patients with stage II CRC.

This article reviews updated information regarding prognostic and molecular aspects of stage II CRC patients who have undergone curative resection, and it also evaluates the significance of such aspects in postoperative surveillance, and the impact on therapeutic strategies.

Conventional Prognostic Factors in Stage II Colorectal Cancer

New American Joint Committee on the cancer staging system

Compatible with other staging systems, the TNM classification for carcinoma of the colon and rectum provides more detail about both clinical and pathologic staging. The 5-year survival rate for patients with CRC is largely dependent on the TNM stage. TNM staging is based on the depth of tumor invasion into or beyond the wall of the colorectum (T), invasion of or adherence to adjacent organs or structures (T), the number of regional lymph nodes involved (N), and the presence or absence of distal metastasis (M).[2]

In the sixth edition of the AJCC/UICC staging systems published in 2002, stage II CRC was subdivided into IIA and IIB. The seventh edition is based on survival and relapse data that were not available for the prior edition, and further substaging of stage II was accomplished, and this is subdivided into IIA (T3N0), IIB (T4aN0) and IIC (T4bN0). T4 lesions are subdivided into T4a (tumor penetrating the surface of the visceral peritoneum) and T4b (tumor

directly invading or is histologically adherent to other organs or structures).[2] The importance of adjacent organs and parietal peritoneum involvement is the key factor for the amendment of stage II CRC by AJCC/UICC staging systems.

Assessment of the number and extent of lymph node involvement

Tumor penetration depth and lymph node metastases have been regarded as significant prognostic determinants for patients with CRC. The number of lymph nodes sampled should be recorded in the assessment of pN. The number of nodes examined from a surgical specimen that is located along the mesocolic border of the colon has been reported to be associated with improved survival.[2,9,10] The presence or absence of lymph node metastasis or the extent of lymph node involvement is one of the most important determinants of prognosis in patients with CRC. Therefore, the presence of nodal metastases provides important prognostic information for therapeutic strategies.[9]

Over the past 60 years, numerous studies have demonstrated that long-term survival following CRC resection is inversely related to the degree of penetration of the primary tumor through the bowel wall and the presence of metastasis in adjacent lymph nodes.[6] The current guidelines from AJCC/UICC recommend that it is important to obtain at least 10–14 lymph nodes in radical colon and rectum resections for accurate staging.[2] Furthermore, several recent studies have suggested that the minimal number of nodes examined should be between 8 and 20.[9–11] There is a consistent risk of a stage III patient being mistakenly classified as stage II when no sufficient lymph nodes are retrieved and then being denied adjuvant chemotherapy. Therefore, an accurate assessment of the tumor lymph nodes in the resected specimen is essential for reducing the risk of under-staging. The 5-year survival rate exceeds 75% in patients with tumors confined to the bowel wall without lymph node involvement, whereas in those with extensive lymph node involvement, it is approximately 30–60%.[9,11,12]

Our recent study[9] has suggested that an increase in the number of tumor-free lymph nodes is clinically important, and this parameter should be taken into consideration in CRC patients without metastatic lymph nodes. Despite the extent of lymph node dissection or the numbers of lymph nodes harvested not greatly improving accurate tumor staging, the increased examined numbers of tumor-free lymph nodes might decrease the incidence of under-staging and provide further therapies for these patients.

Lymphatic, vascular and perineural invasion

Lymphatic and vascular invasion represent crucial steps in the formation of micrometastases and metastases, but some studies have shown venous invasion to be more important than lymphatic invasion, whereas other studies have shown the opposite. Some of the variation in the reported studies probably relates to interobserver variability among pathologists.[13] Vascular invasion by primary tumors may indicate that cancer cells have spread throughout the body, and they have been used as a prognostic factor for predicting recurrence or metastases.[14,15] However, Khankhanian et al[16] suggested that vascular invasion within the bowel wall is not an important prognostic factor among patients with stage II CRC. There are no widely accepted standards for pathological evaluation of vascular invasion. Lymphovascular invasion is considered high risk and an indication for administering chemotherapy, warranted by the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology Version 1, 2010.

Fujita et al[17] reported that perineural invasion status can be used to facilitate the selection of CRC patients for adjuvant chemotherapy and that it should be described in routine pathologic reports. Similarly, the results of our previous study revealed that the presence of perineural invasion might lead to postoperative early relapse in either colonic or rectal cancer. In addition, vascular invasion is not a significant factor in predicting postoperative early relapse of rectal cancer, but it is significant in colon cancer. Perhaps this is due to the more complicated vascular supply in the colon than in the rectum.[18]

Stage II colorectal cancer and indications for treatment

The overall survival in stage II CRC patients is approximately 70–80% at a 5-year interval after radical resection, and in high risk stage II CRC, the clinical outcome is similar to that of patients with stage III disease. Recognition of heterogeneity has led to an increasing focus on the factors that influence prognosis, and also to an intensive search for reliable prognostic markers and markers predictive of a likely response to adjuvant therapy.[11]

Clinical guidelines for management of stage II CRC state that the standard of care is surgical resection alone. However, these guidelines are based on the statement that adjuvant chemotherapy may be considered in cases where there are pathological features of poor prognosis. A wide variety of potential clinical and pathological risk factors for stage II CRC recurrence or metastases have been investigated. The most important pathological risk factors

for predicting the risk of recurrence or metastases are (1) emergency presentation (free bowel perforation or occlusion); (2) detection of lymphatic and vascular invasion in the primary tumors; (3) depth of tumor invasion and adjacent organ involvement; and (4) detection of occult neoplastic cells in lymph nodes, extramural venous invasion, and peritoneal involvement. Other possible factors are much more controversial, such as tumor location (left *vs.* right, distal or proximal to splenic flexure), age, differentiation and sex.[6,11,19]

The widespread use of 5-FU/leucovorin or 5-FU/leucovorin plus oxaliplatin adjuvant chemotherapy regimens for the treatment of patients with stage III CRC has led to a significant improvement in the prognosis of these patients. The key issue in developing treatment strategy is the heterogenous nature of stage II disease, which results in a wide range of postresection prognosis.[11] In contrast to CRC patients with positive lymph nodes (stage III) where adjuvant chemotherapy is beneficial, the use of adjuvant chemotherapy for patients with stage II remains controversial. The International Multicenter Pooled Analysis of Colon Cancer Trials B2 study,[20] which combined data from patients in five separate trials, showed that stage II CRC patients are generally considered to be at low risk for developing postoperative relapse and do not show any statistically significant benefit of 5-FU/leucovorin combination over surgery alone. Recently, an American Society of Clinical Oncology panel[21] reviewed the available literature-based evidence regarding adjuvant therapy in stage II patients. The data revealed that although there was evidence of improvement in disease-free survival with adjuvant therapy, the direct evidence from randomized controlled trials did not support the routine use of adjuvant chemotherapy for patients with stage II CRC because of insignificant improvement in overall survival.[21] Although available evidence does not support the routine use of adjuvant chemotherapy in all patients with stage II CRC, therapy should be considered for poor prognosis patients who possess such risk factors, as previously described.

In contrast, the National Surgical Adjuvant Breast and Bowel Project showed that the relative benefits were largely the same for patients with stage II and stage III tumors for both disease-free survival and overall survival.[22] These data were supported by a meta-analysis from the Mayo Clinic that was derived from 3341 patients with both node-negative and node-positive colon cancer.[23] A report from the Quick and Simple and Reliable (QUASAR) study compared adjuvant 5-FU-based therapy with observation in 3238 CRC patients (91% Dukes' B) from 150 centers in 17 countries. The results of the QUASAR study are an important advancement in understanding the value of adjuvant 5-FU-based chemotherapy, at least for some stage II patients.[24] Therefore, overall, these studies suggest that chemotherapy can lead to a similar incremental benefit of survival for stage II patients (node-negative CRC), as demonstrated for stage III patients (node-positive CRC).

The Multicenter International Study of Oxaliplatin 5-FU/leucovorin in the Adjuvant Treatment of Colon Cancer trial, which randomized patients to receive either infusional 5-FU or infusional 5-FU with oxaliplatin (FOLFOX4), showed a significant improvement in 3-year disease-free survival for more than 1100 patients (node-negative *vs.* node-positive: 40% *vs.* 60%) who were treated with FOLFOX.[25] Growing evidence that the prognosis of certain stage II CRC patients with unfavorable prognostic factors can be improved by adjuvant chemotherapy indicates the need to identify novel predictive factors to guide the identification of stage II CRC patients who are likely to experience metastases and recurrence. Consequently, to improve the clinical outcome of patients with stage II CRC, further studies are required to identify novel panels of molecular and biochemical markers that may be used to predict benefit of adjuvant treatment in stage II CRC.

Molecular (Biological) Markers

Two distinct mutational pathways have been identified that result in CRC. The tumor suppressor pathway, also termed the chromosomal instability pathway, accounts for approximately 85% of all colorectal carcinomas and most sporadic colorectal carcinomas. These genetic changes result in the mutational activation of oncogenes coupled with mutational inactivation of tumor suppressor genes.[26] The second mutational pathway, characterized by the inactivation of both alleles of one of the DNA mismatch repair genes, accounts for approximately 15% of all CRCs. The DNA mismatch repair gene results in variations in the length, and therefore, instability of short tandem DNA sequences known as microsatellites. A number of markers determined from several retrospective studies can now define patients with a high risk factor for metastases or recurrence of both stage II and III disease.[27] We have critically reviewed the data examining potential molecular markers of prognosis and response to therapy for patients with stage II CRC as follows.

Microsatellite instability

Defects in mismatch repair lead to high-frequency MSI in CRC. One of the most extensively investigated molecular markers is MSI, which occurs in approximately 15% of CRCs. High-frequency MSI tends to

be associated with distinct clinical and pathological tumor characteristics, e.g. located proximal to the splenic flexure, mucinous cell type, peritumoral lymphocytic infiltration, poor differentiation, and diploidy.[10] MSI has been investigated in a number of studies as an independent marker of prognosis and response to therapy, and it was found to be a predictor of favorable outcomes compared with microsatellite stability tumors at each stage, with the 5-year survival rate being significantly better in those patients whose tumors exhibited high-frequency MSI.[28]

Fluorouracil-based adjuvant chemotherapy benefits patients with stage II or III colon cancer with microsatellite-stable tumors or tumors exhibiting low-frequency MSI, but not those with tumors exhibiting high-frequency MSI.[28] Halling et al[29] studied 508 patients with resected stage II and III CRC and found that high-frequency MSI was an independent predictor of improved survival and time to recurrence. Parc et al[30] observed a significantly better disease-free survival rate in patients whose tumors presented as MSI, and they also observed a trend for the probability of a longer overall survival period. Adjuvant chemotherapy may be inappropriate and of little or no benefit for stage II CRC patients presenting with high-frequency MSI, because the prognosis for these patients is already good. In contrast, the National Surgical Adjuvant Breast and Bowel Project did not find that MSI was a predictive marker for response to 5-FU; patients with MSI and microsatellite stability were found to benefit equally from the use of adjuvant chemotherapy.[31] Currently, the role of these predictive markers is being tested in stage II patients in a Gastrointestinal Intergroup study conducted by the Eastern Cooperative Oncology Group (E5202), which is addressing their potential as predictors of chemotherapy response.

Overexpression or lack of expression of the "deleted in colorectal cancer" gene

Another leading candidate marker is the allelic loss of chromosome 18q, which is associated with tumor progression and is related to the "deleted in colorectal cancer" gene. The loss of one allele is referred to as a loss of heterozygosity. The most promising candidate markers at present are MSI and allelic loss of chromosome 18q. Halling et al[29] did not find that allelic deletion of 18q was prognostic in stage II and III CRC. However, several studies have demonstrated that patients with retention of both 18q alleles have a more favorable outcome after adjuvant 5-FU-based chemotherapy in stage II disease.[32–34] Lanza et al[33] observed that stage II cancer patients with intact 18q alleles have an excellent clinical outcome with a 5-year disease free survival of 96%. Similarly, Zhou et al[34] found that the 5-year disease free survival of patients with stage II CRC was 100% for those with no allelic balance compared with 58% for those with allelic imbalances of chromosomes 8p and 18q. Collectively, these data support the role of 18q loss of heterozygosity as a prognostic indicator in curable disease, and this may identify patients who will benefit from adjuvant chemotherapy in stage II CRC.

Thymidylate synthase and dihydropyrimidine dehydrogenase

Thymidylate synthase is a key enzyme in DNA synthesis, and a number of enzymes affect the efficacy of 5-FU. The enzyme dihydropyrimidine dehydrogenase metabolizes and inactivates 5-FU in the liver. Kornmann et al[35] investigated the association of thymidylate synthase and dihydropyrimidine dehydrogenase mRNA levels with recurrence-free survival in patients with stage II and III CRC who were receiving adjuvant 5-FU-based chemotherapy. They found that high levels of thymidylate synthase and dihydropyrimidine dehydrogenase were associated with resistance to 5-FU in advanced colorectal cancer. Thymidylate synthase mRNA levels may be a useful marker to predict the time to recurrence in patients with colorectal cancer who are receiving adjuvant 5-FU treatment.[35] However, these findings need to be further evaluated in large prospective studies that may identify patients unlikely to benefit from 5-FU but who may benefit from an alternative chemotherapeutic drug. Additional studies with consistent methodology are required to define the precise prognostic value of thymidylate synthase and dihydropyrimidine dehydrogenase.

Circulating tumor cells and micrometastases

During metastasis, the cells escape the host's defense mechanism, finally forming a new metastatic lesion with tumor cells spreading through the blood stream and lymphatic system. The importance of circulating tumor cell emboli in the spread of metastases has been reported.[36,37] Because dissemination of neoplastic cells is the main determinant of distant metastases or recurrence and cancer-related death, detection of micrometastases and circulating tumor cells in patients undergoing surgery for cure of malignancy remains a challenge for oncologists. Undetected micrometastases can contribute to the failure of primary treatment. Therefore, the identification of occult metastases in patients with early stage cancer could have a substantial clinical impact on the optimal therapy and prognosis for patients with CRC. However, such neoplastic cells may be present in the bloodstream in very low numbers

and may not be detected by conventional methods. Among the current possibilities, one of the most compelling diagnostic methods is the development of a highly sensitive molecular diagnostic procedure for tissues and biological fluids, especially peripheral blood.[36,37]

Some studies have shown that micrometastases do occur and molecular detection of micrometastases is a prognostic tool in stage II CRC.[8,36,38] Koch et al[8] showed the prognostic significance of tumor cells detected in blood samples of patients with stage II CRC using cytokeratin-20 reverse transcription-polymerase chain reaction. Likewise, Lloyd et al[38] showed that detection of marker-positive cells by immunobead reverse transcription-polymerase chain reaction in peritoneal lavage fluid taken during laparotomy was a significant risk factor for reduced survival after curative resection. Consistent with these studies, positive circulating tumor cells are a potential auxiliary tool for conventional clinicopathological variables for the prediction of postoperative relapse in stage II CRC patients who have undergone curative resection.[36] Conversely, some studies have shown that antibodies against cytokeratins and carcinoembryonic antigen have been used to detect micrometastases but they disagree about the prognostic significance of such a finding. Simultaneously, several recent studies have reported conflicting results regarding the prognostic value of CTCs.[39,40] Differences in the choice of antibody, technique of staining procedures and interpretation may explain in part these differing results.[41]

Consequently, further studies are required to determine whether the introduction of adjuvant chemotherapy for stage II CRC patients with positive CTCs is appropriate.[36] Monitoring of circulating tumor cells in stage II CRC patients might be advantageous in postoperative surveillance and might also be useful for evaluation of further adjuvant therapeutic strategies after surgery. However, large scale and long-term clinical follow-ups are warranted to address the clinical significance of stage II CRC for adjuvant chemotherapy.

Conclusion

Optimal tailored chemotherapy aims to treat stage II CRC patients under effective and safe guidelines. The relevant data are contradictory in several aspects, although a prognostic significance is suggested for many of the molecular biological alterations that are causally associated with the genesis of CRC. Up-to-date, randomized controlled trials and meta-analyses have uniformly failed to definitively detect a survival benefit for adjuvant chemotherapy in stage II CRC. Nonetheless, there remains no biological or clinical reason that the clinical behavior of stage II tumors should be different from that of stage III tumors. Combining conventional prognostic markers and molecular markers as predictive factors may warrant clarifying the guidelines for management of this disease in relation to recommendation for further adjuvant chemotherapy.

In the near future, more well-designed and larger clinical trials may be needed to support the prognostic significance of circulating tumor cells with stage II CRC. In addition, it will be necessary to analyze clinical data from multiple institutions to develop more sensitive, simpler, and specific criteria and biomarkers for detecting patients with a probable high risk of post-operative relapse.

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