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Molecular Analyses in Peritoneal Metastasis from Colorectal Cancer: A Review-An English Version

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Abstract

Despite a trend showing continued improvement in survival by combing targeted agents in colorectal cancer, the improvement was limited, and clinically meaningful benefits were not achieved in peritoneal metastasis. The role of cytoreductive surgery (CRS) and proportion of the benefit from hyperthermic intraperitoneal chemotherapy (HIPEC) have been questioned. The PRODIGE 7 study aimed to assess the specific contribution of HIPEC to the survival benefit of peritoneal metastasis from colorectal cancer (CRC-PM) by grouping CRS alone versus CRS with oxaliplatin-based HIPEC, but failed to show any survival improvement. Of these criticisms, oxaliplatin resistance was suggested as the main cause of the negative result. In this regard, the relative resistance to oxaliplatin in consensus molecular subtype 4 colorectal cancer (CRC) is of great interest. Recent treatments for metastatic CRC have gradually moved to precision medicine based on individual biological information through high-throughput technology such as next generation sequencing. This review aimed to provide an overview of the current status of studies reporting the molecular knowledge of CRC-PM.

Keywords

peritoneal metastasis, colorectal cancer, genomics, transcriptomics

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Introduction

Colorectal cancer (CRC) is one of the most common cancers globally and in South Korea[1]. Surgical treatment and neoadjuvant and/or adjuvant chemotherapy can substantially improve survival outcomes in patients with stage I-III CRC[2-5]. However, despite a trend showing continued improvement in survival by combing targeted agents, the improvement was limited, and clinically meaningful benefits were not achieved[6]. In particular, peritoneal metastasis from CRC (CRC-PM) is found in 5%-15% of CRC cases, with the poorest prognosis compared to other sites of metastases, such as the liver or lung[7-9]. A shorter overall survival rate of 30%-40% has been reported in previous studies[10,11]. From the perspective of treating CRC-PM, relatively firm evidence from randomized controlled trials and recently updated meta-analyses suggests that cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) can improve oncologic outcomes in some patients[12-14]. However, with the advancement of systemic chemotherapy, the role of CRS and proportion of the benefit from HIPEC have been questioned. The PRODIGE 7 study aimed to assess the specific contribution of HIPEC to the survival benefit of CRC-PM by grouping CRS alone versus CRS with oxaliplatin-based HIPEC[15]. Although many criticisms of this study were raised and suggest that HIPEC should not be abandoned, the authors failed to show any survival improvement in patients treated with CRS plus HIPEC to that of CRS alone. Of these criticisms, oxaliplatin resistance was suggested as the main cause of the negative

Table 1. Ten Most Frequently Identified Mutation in Primary Colorectal Cancer and CRC-PM.

Gene	Hypermutated cancers (%)	Gene	Non-hypermutated cancers (%)	Gene	CRC-PM (%)
ACVR2A	63	APC	81	APC	44
APC	51	TP53	60	TP53	54
TGFBR2	51	KRAS	43	KRAS	45
BRAF	46	TTN	31		
MSH3	40	PIK3CA	18	PIK3CA	13
MSH6	40	FBXW7	11		
MYO1B	31	SMAD4	10	SMAD4	22
TCF7L2	31	NRAS	9	BRAF	15
CASP8	29	TCF7L2	9		
CDC27	29	FAM123B	7		

result. Recently, one study showed that CRC-PM forms a near-homogenous consensus molecular subtype (CMS) 4 (29/34 primary tumor regions, 58/59 peritoneal metastases), which is generated by transcriptional profiling[16]. The authors suggested that the relative resistance to oxaliplatin in CMS4 CRC could originate from these virtually homogenous CMS4 entities with CRC. In addition, they showed that glutathione synthesis might be associated with a major oxaliplatin resistance pathway, which is highly expressed in CRC-PM-derived organoids[16]. Similar to the abovementioned studies, "-omics" data provide a new opportunity to evolve the precision oncologic paradigm in the treatment of CRC-PM.

Recent treatments for metastatic CRC (mCRC) have gradually moved to precision medicine based on individual biological information through high-throughput technology such as next generation sequencing (NGS). In 2012, The Cancer Genome Atlas network conducted genome-wide analyses of 276 samples via exome sequencing, DNA copy number variation (CNV), DNA methylation analysis, and mRNA and miRNA expression[17]. In addition, as mentioned above, CMS classification using an RNA expression-based system is regarded as the most consistent not only biologically but also clinically relevant disease subtyping[18]. Better knowledge of this advanced molecular information might help predict the prognosis and benefit of specific drugs, aid patient selection, and assist advance effective drug development[19]. Studies to unravel the molecular characteristics of CRC-PM involve genomics, transcriptomics, and proteomics. Understanding of the molecular features of CRC-PM has not yet reached a level that can influence clinical management on a level comparable to other sites of metastasis of CRC or different types of malignancy. This review aimed to provide an overview of the current status of studies reporting the molecular knowledge of CRC-PM.

Genomic Analysis

Genomics is the study of human genes and chromosomes and an interdisciplinary field of biology focusing on the structure, function, comparison, and mapping of genomes. Various methods have been developed for DNA sequencing. It was invented in the mid-1970s by Fredrick Sanger, Alan Maxam, and Walter Gilbert, and sequencing has been a cornerstone of genomics[20,21]. Recently, NGS has been developed and tremendously empowered researchers to gain insights into various diseases.

In the genomic analysis of CRC-PM, many studies have focused on mutation analyses, with a minority of cases being analyzed for CNV. The variation in methods for mutation identification is mainly based on single-gene analysis or NGS. Most studies were relatively small (less than 100 cases), with some exceptions.

In primary CRC, The Cancer Genome Atlas network reported that 16% of CRC samples were hypermutated, three-quarters had microsatellite-instability-high (MSI-H), and one-quarter had mismatch repair gene mutations[17]. Excluding these hypermutated samples, they showed that 24 genes were frequently mutated, in order from highest frequency, APC, TP53, KRAS, TTN, PIK3CA, FBXW, SMAD4, etc. (Table 1). In addition to mutation analyses, they grouped CRC by hypermutation status and recurrent alterations as WNT (APC, CTNNB1, SOX9, TCF7L2, DKK, AXIN2, FBXW7, ARID1A, and FAM123B), PI3K and RAS-MAPK (IGF2, IRS2, PIK3R1, PIK3CA, PTEN, KRAS, NRAS, BRAF, ERBB2, and ERBB3), TGF-β (TGFBR1, TGFBR2, ACVR2A, ACVR2A, ACVR1B, SMAD2, SMAD3, and SMAD4), and p53 (TP53 and ATM) pathways by adding CNV and mRNA expression information[17]. Basically, the concordance of key driver mutations (APC, KRAS, BRAF, PIK3CA, SMAD4, and p53) between primary CRC and mCRC has been known to be very high[22]. They reported that the median concordance was 93.7% for KRAS, 99.4% for BRAF, 93% for PIK3CA, and

92.9% for TP53. However, the absolute concordance for more than one marker decreased as the number of markers included was increased. For example, KRAS and BRAF mutations showed 92%-95% concordance, which decreased to 87% with comprehensive RAS mutation analysis (KRAS, NRAS, and HRAS). This phenomenon was more prominent in studies that used NGS[22]. Although the frequencies of the mutations in CRC-PM were relatively variable, the mutation patterns were similar to those of primary CRC: TP53 (median 54%; min-max 33%-75%), KRAS (45%; 20%-58%), APC (44%; 31%-57%), SMAD4 (22%; 15%-29%), BRAF (15%; 6%-36%), and PIK3CA (13%; 9%-14%)[23].

To date, RAS and BRAF mutation status are the most important biomarkers for the current treatment of mCRC[24]. A recent study also showed that immune checkpoint blockade (pembrolizumab) has clinical benefits in MSI-H or mismatch-repair-deficient tumors[25]. Of note, the RAS/RAF protein is a downstream messenger of the epidermal growth factor receptor that regulates cancer cell proliferation, apoptosis, and angiogenesis. EGFR is expressed in approximately 85% of patients with mCRC and has been validated as a relevant therapeutic target in these patients[26]. As presented above, the proportion of KRAS mutation (20%-58%) and BRAF mutation (8%-13%) in patients with CRC-PM was relatively consistent[27-30], with some exceptions in the BRAF mutation proportion (25%-36%)[31,32]. Similarly, in primary CRC, KRAS mutations were reported in 35%-40% of primary CRC cases, whereas NRAS and HRAS mutations were reported in less than 3% and 1% of primary CRC cases, respectively[33]. BRAF mutation was observed in 5%-10% of primary CRC cases and was mutually exclusive of a KARS mutation[34,35]. The prognostic role of KRAS/BRAF mutation was not clear in the context of the treatment of CRC-PM. Many previous studies, mainly based on single-gene analyses, have reported conflicting results[27-30,36]. In addition, landmark randomized controlled trials comprised a very small number of CRC-PM cases from their entire enrolled cases[24,25]. A few studies used various sized targeted NGS sequencing panels (50-500 genes), with no detailed exploratory analysis. Mutations in predefined genes of previously reported or known genes were included and analyzed. Interestingly, Yaeger et al. reported a genomic analysis of over 500 mCRCs and a few genomic differences between primary CRC and mCRC[37]. They added that tumor laterality of primary CRC (right-sided vs. left-sided) has a survival impact on their mCRC, and that KRAS, BRAF, PIK3CA, AKT1, RNF43, and SMAD are enriched in right-sided CRC. However, owing to the small sample size and undetailed subgroup analysis, these findings are not clearly presented with respect to CRC-PM[37]. Similarly, Baratti et al.[30] reported that BRAF mutations and right-sided primary CRC have an adverse prognostic impact on CRC-PM. Interestingly, they

showed that targeted NGS included 50 genes in a limited subgroup (68 of 156 patients); although KRAS, NRAS, BRAF, and PIK3CA were more frequent in right-sided primary CRC, only APC might be underrepresented (36.8%). Considering that APC mutations occur relatively early in carcinogenesis and are found in 70%-80% of CRC cases, they were inversely correlated with CRC-PM compared to other mCRCs. A recent study provided important insights into CRC-PM intratumor and intrametastatic heterogeneity. Siesing et al.[38] showed the results of deep targeted DNA sequencing of chemotherapy-naïve tumors from seven patients with synchronous CRC-PM who underwent CRS and HIPEC. In their study, 88 samples (5-19 per patient) representing primary tumors, lymph nodes, tumor deposits, peritoneal carcinomatoses, and liver metastases were analyzed. In agreement with the results of Yaeger and Baratti[30,37], MSI and key driver gene mutations such as KRAS, APC, and TP53 were homogenously distributed throughout the samples. However, passenger mutations and less common mutations were more heterogeneous intra- and inter-patient.

Few studies have reported CNV analysis in CRC-PM. As described above, genetic alterations have been thoroughly described by The Cancer Genome Atlas network for primary CRC and Mendelaar et al. for mCRC[17,39]. The most frequent CNVs in the CRC-PM were similar to those found in primary CRC. In 2004, Diep et al.[40] analyzed 10 cases of primary CRC and 7 peritoneal carcinomatosis using comparative genomic hybridization. They reported that 5p and 12p were more commonly present in CRC-PM. Similarly, Kleivi et al.[41] also found increased expression of 5p genes in CRC-PM compared with primary CRC and liver metastases.

Transcriptomic Analysis

Gene expression-based molecular classification is widely accepted as a relevant source of disease subtyping[42]. Cancer research is currently the most important application area in this field. However, to date, fewer than 50 CRC-PM samples have been analyzed using microarray technology[41,43-45]. In CRC-PM, genes involved in the WNT signaling pathway were highly expressed. As stated above, genes located in 5p were highly expressed in CRC-PM[41].

In an effort to describe the behavior of CRC and guide more specific treatment, the CRC Subtyping Consortium unified the previous molecular classification system, which was based on gene expression data, into a new consensus system with four distinct classifications: CMS1, CMS2, CMS3, and CMS4[18]. The CMS classifications were based on transcriptomic, epigenomic, genetic, microenvironmental, and clinical characteristics. CMS1 is immunogenic and hypermutated. CMS2 tumors are highly expressed in the Wnt-β-catenin pathway and have the highest overall survival rate.

CMS3 features a metabolic cancer phenotype, and CMS4 cancers have a strong stromal gene signature with the worst survival. Notably, CMS4 is known to have the worst prognosis and benefits least from systemic chemotherapy[18,46]. CMS4 is characterized by the TGF-β/Smad pathway that ultimately leads to the induction of epithelial-mesenchymal transition (EMT) that enables stationary CRC cells to lose their cell to cell adherence and acquire mesenchymal properties that are essential for invasion and peritoneal metastasis. In this regard, Ubink et al.[31] reported that CMS4 is significantly enriched in primary CRC with synchronous peritoneal metastasis using RT-PCR, which predicts the probability of CMS4 using four markers[47], compared with unselective stage I-IV primary tumors (60% vs. 23%) in the original study[18]. Furthermore, heterogeneity in CMS4 between primary CRC and metastatic lesions was found to be considerably high; intrapatient heterogeneity was >50%. Interestingly, this heterogeneity was observed in all the patients with more than one metastasis[31]. The authors extended their study to 35 primary CRC and 59 paired CRM-PMs from 12 patients, using RNA sequencing[16]. First, they showed that patients and/or tumor genetic characteristics appear to be more decisive factors of gene expression variation than the different tissue microenvironments. Second, they identified 15 hallmark pathways that were expressed higher in CRC-PM than in their corresponding primary CRC samples. TGF-β signaling, angiogenesis, complement activation, and EMT were significantly enriched in the CRC-PM. Third, a near-homogenous classification of all tumors in the CRC-PM cohort as CMS4 (29/34 primary tumor regions, 58/59 peritoneal metastases) is shown. Of the 12 patients, the sole primary tumor was classified as CMS2, but 11 peritoneal lesions from a total of 12 peritoneal metastatic lesions were classified as CMS4. Finally, they showed that glutathione synthesis was a major oxaliplatin resistance factor in the treatment of CRC-PM, which explains why oxaliplatin-based HIPEC has a negative result in the PRODIGE 7 study[15]. Based on these results, they proposed that the addition of drugs that inhibit glutathione synthesis to an oxaliplatin-based HIPEC regimen subsequently lowers the reductive capacity of tumor cells, which may enhance treatment efficacy and improve survival in CRC-PM.

Conclusions

The current status of knowledge based on the molecular research of CRC-PM has many shortcomings and huddles to use in diagnosis, prognosis, and treatment. In many studies, good quality-omics data were reported together with results from pCRC, other metastatic sites (mainly the liver, lung, and lymph node), and even different disease entities such as pseudomyxoma peritonei and appendiceal malignancy. Additionally, the number of cases tested was small and varied substantially. Another important consideration when analyzing molecular data (genetic, epigenomic, CNV, and transcriptomic data) in a clinical situation is whether the analyzed data are from representative cohorts with adequate clinical data. In fact, the sample analyzed in CRC-PM was collected at the time of surgery, whereas samples from patients not undergoing surgery were not included for further analysis. Since the number of patients attempting CRS or CRS with HIPEC is relatively small, this issue should be considered.

Mutation analysis is an important pillar of the treatment guide and is more consistent and robust. Mutations that have been clinically actionable and targeted, such as KRAS mutations, BRAF, and MSI status, are generated from mutation analysis. In addition, high concordance was observed across multiple CRC key driver gene mutations between primary CRC and metastatic lesions, especially in the liver and lungs. However, this remarkably high concordance was confirmed in CRC-PM. Different results from the liver and lungs may explain the significantly worse prognosis in this patient group. Using NGS technology, potentially targetable mutations, such as PI3KCA, AKT, LKB1, KIT, MET, and ERBB2, have been newly discovered in CRC-PM[17,37]. In transcriptomic research, CRC-PM was identified to be a near-homogenous CMS4 classification, which is known to be strongly resistant to oxaliplatin-based chemotherapy. These results suggest a new need for the clarification of complex downstream signaling pathways derived from transcriptomic or proteomic studies of CRC-PM. Finally, building a representative patient cohort and standardized sample collection, processing, and analysis should be considered to achieve an inter-research comparison.

Conflicts of Interest
There are no conflicts of interest.

Author Contributions
Chang Hyun Kim: conception and design of the study, drafting and revising the study, and final approval of the version to be submitted.

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