Genomics of peritoneal surface malignancies

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Abstract

Peritoneal malignancies and metastasis are traditionally approached as a terminal disease, however with multiple lines of clinical therapy; long-term survival can be achieved in selected patients using aggressive cytoreduction and hyperthermic intraperitoneal chemotherapy. This is especially true for *Pseudomyxoma peritonei* from appendiceal neoplasms, peritoneal mesothelioma and peritoneal metastasis from colorectal cancer. In this article, we discuss the nature of genomic alterations in these three peritoneal malignancies and their potential as prognostic and therapeutic markers in clinical decisions. Genomic characterization of malignancies using technological advances including what is now widely used and accepted next-generation genomic sequencing methods has identified genomic anomalies (*i.e.* mutations, epigenetic modifications, transcription and expression changes in RNA) which is used for targeted therapy, prognostication, surveillance and prediction of response to therapy.

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Background

Ongoing efforts to further characterize the genomics of peritoneal malignancies and metastasis remain essential. Although the appendix is considered as a part of the colon, its cancer genomic landscape is very different from colorectal cancer, suggesting that much like the variation in right and left-sided CRC, regional variation in gastrointestinal tract (GIT) tissues contributes to the unique disease phenotype. Equally, in Pseudomyxoma peritonei (PMP) of appendiceal origin, the most common gene mutations are in KRAS and GNAS. In addition, unlike CRC, DNA microsatellite instability and mutations in housekeeping DNA repair genes are typically rare (approximately 3%) and is therefore not a common phenotype of PMP. Research and discovery are still needed on genomic alterations driving peritoneal metastasis from CRC, although there is some evidence that BRAF mutations are associated with higher incidence of peritoneal metastasis. A better understanding of disease pathways linked with genomic alteration would contribute to our clinical goals of personalized medicine.

Introduction

Global genome sequencing and characterization efforts have transformed our understanding of cancer biology, pathogenesis, and etiology. Knowledge of the molecular alterations that drive cancer development-including the genome, transcriptome, methylome and miRNAome alterations can be applied, in principle, to develop integrated approaches for personalized cancer treatment.[1] Reduction in the costs of genomic techniques is making it possible to bring personalized medicine to the bedside.

Historically peritoneal metastases from GI cancers were regarded as a terminal condition with survival ranging from weeks to a few months.[2] However, significant prolongation of survival has achieved with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with limited peritoneal metastases from appendiceal and colorectal cancer over the last two decades. CRS typically involves radical surgical removal of all macroscopic tumor with peritonectomy and multiple visceral resections, and HIPEC involves administration of heated high dose chemotherapy intraperitoneally, usually mitomycin-c or oxaliplatin.[3,4] The genomics of peritoneal carcinomatosis (PC) however is still poorly understood. Early genomic profiling studies do suggest unique gene expression patterns may determine whether colorectal and high-grade appendiceal adenocarcinoma will spread into peritoneal tissues, towards the liver, or both.[5] A better understanding of genes and pathways regulating tumor spread to the peritoneum may allow for the development of novel, targeted molecular agents. Another challenge is to identify clinically relevant biomarkers for improving diagnosis, surveillance, prognostication and pre-

diction of response to therapy. Genomic markers such as microsatellite instability (MSI), KRAS, and BRAF mutations have been used in recent years to predict response to chemotherapy in advanced CRC.[6-8] Better genomic characterization of peritoneal carcinomatosis would similarly allow for personalized approach by identifying patients that will benefit most from aggressive surgical treatments such as CRS and HIPEC.

In this article, we review the current literature on genomics for pathophysiology and clinical care of patients with common peritoneal surface malignancies encountered by surgeons including peritoneal metastasis from CRC, appendiceal neoplasms and peritoneal malignant mesotheliomas. Over the past decade, there has been tremendous advancement in the next generation sequencing technologies, which imparts more accuracy to the scientific data collected in the past decade. The articles reviewed in this manuscript were selected by the authors for their reliability of the data, genomic sequencing techniques, cellular enrichment approaches, impact on clinical practice and relevance to the clinical outcome.

Pathophysiology of peritoneal dissemination

Peritoneal dissemination is a sequence of events that results in a cascade of cellular changes contributing to metastasis. It results from the complex molecular interaction between tumor cells and the peritoneum. The tumor cells are thought to detach from their primary tumor, gain motility, and eventually adhere to the peritoneal surface. Tumor cells ultimately invade the mesothelium, proliferate and form peritoneal metastasis (PM). These events do not necessarily occur in isolation, but rather describe a continuous and interdependent process.[9,10]

At the molecular level, change in phenotype of epithelial cells occurs via a process called epithelial-mesenchymal transition (EMT). EMT allows a polarized epithelial cell, which normally interacts with the basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components.[11] Peritoneal metastasis from CRC have higher rates of EMT, and primary CRC with EMT dysregulation have higher rates of reoccurrence.[12,13] Down-regulation of cell–cell adhesion molecules, such as E-cadherin also has been reported in EMT and is associated with the dedifferentiation, progression, and metastasis of colorectal cancer.[14,15]

Table 1. Common genetic mutations in PMP of appendiceal origin and peritoneal mesothelioma.

Cancer type	Mutation	Approximate frequency
LAMN and PMP[21-26]	KRAS	53-100 %
	GNAS	40-63 %
	MCL1 & JUN1 amplification	30 %
	MSI, p53	Uncommon
Peritoneal mesothelioma[37,38]	BAP1	55-69 %
	NF2	23%
	SETD2	15%
	DDX3X	15%

High variation in mutation rates is likely from a small number of patients, the difference in cell enrichment methods and genome sequencing techniques in individual studies.

The genomics of peritoneal malignancy

Technological advances such as NGS, comparative genomic hybridization (CGH) arrays and single-nucleotide polymorphism (SNP) arrays now provide fundamental insight into the complex genomic landscapes of peritoneal malignancies. It is now possible to perform a genome-wide analysis that helps us to understand the biology of different tumor types. In this section, we will attempt to dissect the genomics of peritoneal surface malignancies based on major disease pathology namely PMP, peritoneal malignant mesothelioma and colorectal cancer.

Pseudomyxoma peritonei genomics

Pseudomyxoma peritonei is an extremely rare condition characterized by progressive accumulation of mucinous ascites and tumor implants throughout the peritoneum with an estimated incidence of 1-2 per million per year.[16] It originates most frequently from the mucinous tumors of the appendix and less frequently from the ovaries.[16] Appendiceal mucinous tumors include a spectrum of tumors, ranging from adenoma to low-grade appendiceal mucinous neoplasms (LAMN) to mucinous adenocarcinoma. Classical PMP usually occurs in association with LAMNs.[17]

Although the appendix is considered a part of the large intestine, the genomics of LAMN and PMP differ significantly from CRC. Genome-sequencing efforts are lagging for PMP due to issues with low cellularity of these tumors, which makes it harder to extract enough DNA material for sequencing. Also, the relative rarity of this disease has limited genomic characterization of these tumors. Recent genomic sequencing efforts in small series have shown that KRAS and GNAS gene mutations appear to be the most prominent players. KRAS gene is a proto-oncogene that encodes a small GTPase transductor protein called KRAS. KRAS is involved in the regulation of cell division as a result of its ability to relay external signals to the cell nucleus.[18] Somatic mutations in KRAS gene are seen in various types of cancer and CRC, its mutation frequency can be as high as 45%.[19] Like CRC, KRAS gene mutations in codon 12 & 13 resulting from amino acid substitution have been reported to be most common in LAMNs. GNAS encodes for stimulatory G protein alpha subunit. Its mutation was originally found in pituitary tumors, intraductal papillary mucinous neoplasms of the pancreas (IPMN), gastric, and intestinal adenomas as well as CRC.[20] Prevalence of KRAS & GNAS mutations in LAMN and PMP tumor samples has been reported in various studies with high variability ranging from 53-100% & 40-63% respectively (Table 1).[21-26] Differences in cell enrichment methods, genomic sequencing techniques, regional variation and small number patients in most of these studies may have contributed to this variation. Alakus *et al.* reported KRAS and GNAS mutations in 10/10 and 9/10 tumor samples respectively, which is quite higher than previous studies.[20] It may be quite possible that due to hypocellular nature of this disease, prevalence of these mutations may have been underestimated in other studies. However, APC and p53 mutation are uncommon in PMP of appendiceal origin in contrast to colon cancer.[20] High-level microsatellite instability (MSI) is also rare (3%) in appendiceal cancers, whereas approximately 15% of colorectal carcinomas (CRCs) display high-level MSI.[27,28] Sio *et al.* identified MCL1 and JUN1 amplification in 30% of PMP cases using next-generation sequencing assay with Illumina HiSeq2000 platform.[24] MCL1 is a BCL2 family anti-apoptotic gene, and its overexpression may contribute to chemotherapy resistance to 5-fluorouracil, which is given commonly for PMP dur-

ing HIPEC.[29] JUN is a proto-oncogene commonly expressed in gastroenteropancreatic neuroendocrine tumors and squamous cell lung cancers. Both of these may represent novel targets for treatment in the future but require further study.[24]

In addition to driver gene mutations, differential gene expression may also contribute to the pathogenesis of these cancers and the cancers may then exhibit unique clinical outcomes. Roberts *et al*. used exon-array analysis to study differential gene expression in PMP samples *versus* normal colonic mucosa; they identified 27 upregulated and 34 downregulated genes in PMP epithelial tissue compared with normal colonic mucosa.[30] Although their sample population was small (4 PMP samples, 3 normal colonic mucosa), their data demonstrated that gene profiles in PMP are distinct from colon cancer. For the first time, they also developed two immortalized PMP cell lines (N14A and N15A). These cell lines can serve as a platform for future pre-clinical anti-tumor drug testing and oncogene discovery in PMP. PMP is chemoresistant, and there is a clear need to improve the effectiveness of current chemotherapy regimens.[30] Levine *et al.* used microarray analysis to study gene expression in low-grade appendiceal primary tumors.[31] In this work, a gene signature (139 gene cassette) was established which could prognosticate patients based on their likelihood of benefit from CRS and HIPEC. This was an important milestone as there is significant and unpredictable variability in the clinical outcome within low-grade appendiceal primary tumors. Identification of a high risk *vs*. low-risk subtype for low-grade appendiceal neoplasms based on genomic information may help identify patients most likely to benefit from treatment regimens.[31]

A separate population of appendiceal adenocarcinomas are those characterized by goblet cell features, in these tumors we observe a unique pathology and genomic profile. These tumors typically have different molecular pathways to carcinogenesis and do not have KRAS or GNAS mutations.[25] Given that KRAS mutations are associated with lack of response to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies (Cetuximab, Panitumumab), its absence in appendiceal adenocarcinomas with goblet cell features may allow for targeted anti-EGFR therapy.[32]

Peritoneal malignant mesothelioma

Malignant mesothelioma (MM) is rare cancer that arises from the mesothelial cells that line the serous surfaces (pleura, peritoneum, pericardium and tunica vaginalis). Only 20% to 33% of all mesotheliomas arise from the peritoneum itself; the pleura is the most common site of origin.[33] It is an aggressive tumor with a historically reported median survival of 10 months.[34] However, recently treatment centers that utilize aggressive treatment using HIPEC and CRS recently have shown improved median survival of 67 months.[35] Asbestos exposure is the principal risk factor, however, this exposure is seen in up to 50% of patients with a peritoneal origin of mesothelioma compared to 80% of mesotheliomas with pleural origin.[33,36] Other potential risk factors include prior radiation, viruses (*i.e*. SV40), and exposure to some other naturally occurring fibrous minerals like Erionite (found in mineral ash) or mica (used in drywall).[33,36]

BAP1 (BRCA1 associated protein 1) is the most commonly altered gene in peritoneal malignant mesothelioma.[37,38] BAP1 is a tumor suppressor, which is believed to mediate effects through chromatin modulation, transcriptional regulation, and possibly via the ubiquitin-proteasome system and the DNA damage response pathway. Germline mutations of BAP1 confer familial predisposition for the development of malignant mesothelioma and several other tumors, including uveal and cutaneous melanoma.[39] BAP1 mutations result in loss of nuclear staining by immunohistochemistry in malignant mesotheliomas and can serve to distinguish it from other diagnoses.

With a median of 1.3 mutations per million base pairs, peritoneal MM has a much lower mutation rate than other adult solid tumors; also, copy number alterations are rare.[37] Other less common mutations involve NF2, SETD2 and DDX3X genes (Table 1). CDKN2A mutation which is common in pleural mesothelioma is uncommon in peritoneal mesothelioma (>60% pleural *vs* 8% peritoneal mesothelioma respectively).[38] BAP1, SETD2 and DDX3X genes play an important role in epigenetic regulation. These represent potential therapeutic targets using pharmacologic inhibition of epigenetic modifier enzymes like histone deacetylases (HDAC) and the histone methyltransferase EZH2.[38]

Colorectal cancer genomics

Colorectal cancer (CRC) is one of the most common forms of cancer and the third most common cause of cancer mortality worldwide.[40] Peritoneal metastases represent a common location for cancer recurrence as 10-25 % of CRC patients eventually develop peritoneal metastases (PM), and in up to 25 % of these patients the peritoneum is the only site of metastasis.[41] Patients with peritoneal metastasis from CRC have significantly shorter overall survival than those with other isolated sites of metastases.[42]

Most CRC cases present sporadically (70%), but approximately 30% of cases have some inherited/familial predisposition. Etiologies of most inherited CRCs (25%) are not completely understood, but approximately 5% of these cases are associated with well-characterized inherited CRC syndromes.[43] Examples of some of these well-known cancer syndromes and their associated germline mutations are detailed in Table 2.

Adenoma-carcinoma sequence model

Sporadic CRC evolves from benign to malignant lesions by a stepwise accumulation of somatic mutations over time, a process that was well described by Fearon and Vogelstein in their adenoma-carcinoma sequence model.[44] The first or *gatekeeping* mutations in the colon most often occur in the APC gene. The absence of functional APC leads to inappropriate and constitutional activation of Wnt signaling. This provides a selective growth advantage to epithelial cells, allowing it to outgrow surrounding normal cells. A second mutation such as in KRAS gene (37% of cases), is required for further expansive clonal growth. KRAS mutations lead to the activation of GTPase resulting in incessant transmission of growth response. With the clonal expansion, mutations in genes

Table 2. Characteristic genetic mutations in hereditary CRC syndromes.[43]

Inherited CRC syndrome	Characteristic genetic mutation(s)
Lynch syndrome	MLH1, MSH2, MSH6, PMS2, EpCAM
Familial adenomatous polyposis	APC
Peutz-Jeghers syndrome	STK11
Juvenile polyposis syndrome	SMAD4, BMPR1A

such as PIK3CA, SMAD4, and TP53 accumulate. These mutations are a late event in tumorigenesis and often coincide with the transition of large adenomas into a malignant tumor that can invade locally and metastasize.[45,46]

Molecular pathways for sporadic colorectal cancer

Colorectal tumorigenesis follows at least one of three well-defined molecular pathways. These include chromosomal instability pathway (CIN), mismatch repair (MMR) defect pathway and aberrant DNA methylation leading to epigenetic silencing of genes. Study of the specific germline mutations responsible for familial CRC cases has provided great insights into understanding these pathways. The chromosomal instability (CIN) pathway correlates with loss of APC, which is typically seen in familial adenomatous polyposis (FAP). Inactivation of mismatch-repair genes occurs in Lynch syndrome (inherited mutation) as well as approximately 15% of patients with sporadic colorectal cancer.[46] The loss of mismatch-repair function is easily recognized by the associated epiphenomenon of microsatellite instability.[46] Epigenetic modifications such as aberrant DNA methylation of the CpG-rich *CpG islands* (a cytosine base is followed immediately by a guanine base) in the promoter regions is also commonly seen leading to silencing of gene expression. In sporadic colorectal cancer with microsatellite instability (MSI), epigenetic silencing blocks the expression of MLH1 leading to mismatch repair. These patients often also have a concordant mutation in BRAF.[47] Also, subsets of CRC including those with MSI may have concordant methylation of multiple genes called the CpG island methylator phenotype.[46,48,49]

Hypermutated *vs.* non-hypermutated tumors

The Cancer Genome Atlas (TCGA) project has profiled genomic changes in multiple cancer types.[50] In CRC, analysis of 276 samples with exome sequencing, DNA copy number, methylation analysis as well as RNA and microRNA expression revealed that 16% of CRC were hypermutated (mutation rates of >12 per 10^6). Three-quarters of these were MSI-H tumors, usually with hypermethylation and MLH1 silencing, and one-quarter had somatic MMR gene and polymerase e (POLE) mutations. Among non-hypermutated tumors, colon and rectum cancers were found to have similar patterns of genomic alteration. This major undertaking revealed the complexity of CRC genomics, with a total of 24 genes found to be significantly mutated; 93% of non-hypermutated and 97% of hypermutated cases had a mutation in one or more members of the WNT signaling pathway, with APC gene mutation being the most common. Additional common pathways altered include TGF-β, RTK-RAS and PI3K signaling pathway (Table 3). New findings included recurrent mutations in FAM123B, ARID1A and SOX9. Mutations and amplifications of ERBB2 were observed in a significant percentage of patients. These discoveries carry translational significance as ERBB2 (HER-2) is a significant cancer therapeutic target with antibody trastuzumab.[50]

Clinical significance of microsatellite instability

Generally, 15% of patients with CRC have microsatellite instability (MSI); the majority of these are sporadic and occur due to methylation of the MLH1 gene. Tumors with MSI tend to occur on the right side of the colon, are poorly differentiated, show mucin production and signet ring cells; Overall, these patients have a better prognosis. These tumors are less likely to metastasize to the peritoneum, unlike microsatellite stable tumors with poor differentiation/mucin production/signet ring cells.[49,51]

Patients with MMR mutations do not seem to benefit from 5-Flourouracil (5-FU) based treatment but may benefit from irinotecan.[52] Interestingly, the presence of MMR mutations is also predictive of response to Pembrolizumab (Programmed death-1 blocker) in colorectal cancer.[8] The frequency of MSI in peritoneal carcinomatosis from CRC is likely low but should be tested for mucinous tumors.[12]

Clinical significance of RAS-RAF-MEK-ERK pathway

The Ras/Raf/MEK/ERK signaling cascade is used by growth factors (*e.g.* EGFR) and mitogens to transmit signals from their receptors to regulate gene expression and prevent apoptosis. RAS and BRAF are two components of these pathways that are mutated or aberrantly expressed in CRC.[53]

KRAS encodes a GTPase (RAS), which is a common upstream molecule in this pathway. Mutations in KRAS (primarily at codons 12 and 13) lead to downstream activation of RAS/RAF signaling and are common (35%-42%) and early events in colon tumorigenesis.[54] The BRAF gene encodes a serine-threonine protein kinase that acts as a downstream effector of KRAS signaling and belongs to the RAS-RAF-MEK-ERK pathway. BRAF mutation is seen in approximately 8% of cases with CRC and is nearly always mutually exclusive with mutations in RAS proteins. V600E is the most frequently observed mutation seen in 90% of cases.[55] BRAF mutation is seen in approximately half of these cases with sporadic high-level microsatellite instability (MSI-H) but is not seen in cases Lynch syndrome. CIMP is tightly associated with BRAF mutation in the presence of sporadic MSI-H.[47,56] Patients with a subset of MSI with BRAF mutation have poor survival compared to patients with MSI-H only. Patients with BRAF mutation are also more likely to have peritoneal metastasis (26% *vs*. 14%).[57]

Anti-EGFR antibodies (Cetuximab, Panitumumab) have been shown to improve survival in patients with advanced CRC. EGFR signaling is closely related to Ras/Raf/MEK/ERK pathway and mutation in KRAS, or BRAF downstream leads to lack of response to anti-EGFR antibodies. Thus, mutation testing for both KRAS and BRAF-V600E is recommended in metastatic CRC before initiation of treatment with anti-EGFR monoclonal antibodies.[58,59]

Table 3. Major pathways altered in CRC and percentage alteration in hypermutated *vs.* nonhypermutated tumors.[50]

Pathway alterations	Hypermutated tumors	Non-hypermutated tumors
WNT signaling	92%	97%
TGF-β signaling	27%	87%
PI3K signaling	50%	53%
RTK-RAS signaling	59%	80%

HER-2(ERBB-2) and colorectal cancer

The Cancer Genome Atlas (TCGA) colorectal cancer project identified HER2 somatic mutations or HER2 gene amplification in approximately 7% of CRC patients.[50,60] It is an alternative mechanism for resistance to anti-EGFR monoclonal antibodies in CRC.[61] Preclinical studies have shown that dual targeted therapy using trastuzumab plus tyrosine kinase inhibitors is effective against CRC xenografts with HER-2 amplification.[60] These findings lead to HERACLES trial, which showed that that the combination of trastuzumab and lapatinib is active in patients with HER2-positive metastatic colorectal cancer refractory to chemotherapy and anti-EGFR antibodies. 30% of patients achieved an objective response in this trial.[62] These results show the clinical relevance of HER2 amplification in metastatic colorectal cancer.

Conclusions

Recent genomic efforts are enhancing our understanding of the biology important to peritoneal malignancies and their pathogenesis. Comprehensive learning about various aspects of molecular mechanisms driving cancer development can be applied to develop integrated approaches for personalized cancer treatment. In this article, we aimed to explore the depth of PC genomics. Although still in its infancy, the advancements in genomic research could potentially translate into clinical use.

A limited number of genetic drivers have been identified for appendiceal neoplasms and malignant mesotheliomas, so far. Druggable drivers in CRC include HER2, MSI, KRAS and possibly BRAF also. Some genetic drivers of peritoneal CRC metastases are being identified, but more information is needed. Targeting of genomic alterations could treat only a subset of patients. This problem could potentially be circumvented with a better understanding of different biological processes that are additive to cancer gene mutations, including epigenetic changes. Evolving integrated approaches in systems biology with genomics, epigenomics and big data analysis would further enhance our understanding of the disease leading towards better understanding and treatment outcome.

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