7 Rationale Behind HIPEC/Molecular and Genetic Considerations in HIPEC

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Introduction

Intraperitoneal (IP) chemotherapy combined with hyperthermia is a well-recognized adjunct to cytoreductive surgery (CRS) when used to treat certain types of peritoneal surface malignancies (PSM), either originating from or spreading to the lining of the abdominopelvic cavity. Hyperthermia has long been utilized as a means to improve efficacy in tumor killing as it is selectively cytotoxic to malignant cells in the range of 41–43 °C due to inhibition of oxidative metabolism, producing a lower microenvironment pH in the malignant cell and increased activity of lysosomes [1]. It has been used alone, in combination with systemic chemotherapy, or in combination with radiotherapy. When hyperthermia is used with IP chemotherapy, the result is an improved therapeutic index and efficacy of the agent [2]. Over the past few decades, hyperthermic intraperitoneal chemotherapy (HIPEC) has emerged as a modality commonly employed at the time of CRS for PSM. Though achieving clearance of all gross visible disease at the time of surgery is the mainstay of therapy, the rationale for direct instillation of HIPEC is based on the theoretical benefit that its addition will provide an additive or synergistic anticancer effect on the microscopic or cellular level while avoiding systemic toxicity. The multimodal approach of utilizing CRS and HIPEC in combination has been clinically demonstrated to impact progression-free and overall survival in several disease processes, such as appendiceal and ovarian cancer [3, 4]. However, it is difficult to parse out the individual contributions of the individual components, as most clinical studies examine CRS and HIPEC as a complete package. Moreover, there is great heterogeneity in the application of CRS/HIPEC, as there is no uniform consensus on technique of HIPEC delivery, duration of IP chemotherapy, temperature of hyperthermia, or chemotherapeutic agent utilized. The scientific basis for use of intraoperative HIPEC is anchored mostly in pharmacologic studies, with data generally supporting improved drug penetration/permeability or increased cytotoxicity [5, 6]. The pharmacokinetics and drug profiles of the chemotherapeutic agents are discussed elsewhere in this book. This chapter explores the molecular and genetic rationale of employing HIPEC.

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Carcinomatosis

Molecular Biology of the Peritoneal Metastatic Cascade

Complete comprehension of the biologic nature of peritoneal tumor seeding has been elusive. Understanding the molecular events of carcinomatosis is important in designing a therapy that is both effective and devoid of unnecessary toxicity. Carcinomatosis may be regarded as a continuous and interdependent series of events forming a peritoneal metastatic cascade [7]. It is a multistep process that requires adaptation of the primary tumor as well as mechanisms enabling tumor adhesion and growth [8]. Lemoine et al. have described a set of well-defined steps in the peritoneal metastatic cascade of colorectal cancer, conditional upon communication between tumor cells and the microenvironment on a molecular level. First, an individual cell or clump of cells detach from the primary tumor. Then, the exfoliated cells are subjected to the forces of peritoneal transport, which tends to occur in a clockwise fashion as a result of bowel peristalsis, changes in intra-abdominal pressure with respiratory variation, and gravity. These cells attach to peritoneal surfaces distant from the primary site. Once attached, cells invade the subperitoneal space, and then finally, angiogenesis with resultant proliferation occurs. The molecular events and pathways are summarized in Table 7.1 [9].

Tumor Microenvironment in Carcinomatosis

The peritoneum, consisting of a monolayer of mesothelial cells supported by a basement membrane on connective tissue, is often regarded as the first line of defense in carcinomatosis [10]. The impact of the tumor microenvironment on tumorigenesis in colorectal cancer carcinomatosis was studied by Seebauer et al. by characterizing proliferation, senescence, and neovascularization in primary tumor cells and metastatic cells. Interestingly, metastatic cancer cells demonstrated lower proliferation (Ki-67, PCNA, Cyclin D1) and higher senescence (H3K9me3, $p21^{Cip1}$, CDKN2A) rates than primary cancer cells. This may partially explain the greater resistance of metastatic cancer cells to systemic chemotherapy. The tumor microenvironment of peritoneal carcinomatosis was found to be abundant in natural killer cells, which play a role in tumor growth, dissemination, and recurrence. In addition, the microenvironment was shown to be rich in angiogenic mediators, such as vascular endothelial growth factor A (VEGF-A) [11].

Gene Expression in Peritoneal Metastases

Gene expression in metastatic colorectal cancer has been studied utilizing DNA microarray. Kleivi et al. found that gains of chromosome arm 5p are common in peritoneal carcinomatosis and several candidate genes (PTGER4, SKP2, and ZNF662) mapping to this region were overexpressed [12]. While histopathologic subtype and grade may provide prognostic information in patients with carcinomatosis, the biologic signature of PSM as it relates to prognosis is poorly understood. Genomic analysis of peritoneal metastases from low-grade appendiceal and colorectal cancer was performed by Levine et al., demonstrating three phenotypic clusters with distinct signatures for low-risk appendiceal cancer, high-risk appendiceal cancer, and high-risk colorectal cancer. The signatures not only predicted survival but also highlighted the unique biology of appendiceal cancer compared to colorectal cancer [13]. The same group more recently reported on a 139-gene expression panel that distinguished two molecular subtypes of disseminated mucinous appendiceal neoplasms with statistically significant survival differences. In a validation cohort, the 139-gene panel reproducibly partitioned tumors treated with CRS/HIPEC into subtypes with significant survival differences. These data are exciting and require further independent validation but suggest the potential for genomics to be incorporated into patient selection for CRS/HIPEC in the future [14].

Table 7.1 The peritoneal metastatic cascade

Step in peritoneal metastasis cascade	Molecule or molecular pathway
Detachment from the primary tumor	Spontaneous tumor shedding: E-cadherin ↓ N-cadherin ↑ EMT PC1 and PC2 ↑ Interstitial fluid pressure ↑ Perioperative tumor seeding during surgery
Peritoneal transport	Mucinous ascites Actin microfilament system Lamellipodia, filopodia
Attachment to distant peritoneum	Transmesothelial dissemination: ICAM-1 ↑, PECAM-1, VCAM-1 ↑ TNF-α, IL-1β, IL-6, IFN-γ β1 integrin subunit CD43, CD44 Hyaluronan Translymphatic dissemination: Lymphatic stomata Milky spots
Invasion into the subperitoneal space	Rounding of mesothelial cells: HGF/SF ↑ c-met ↑ Tumor-induced apoptosis Fas ligand/Fas Adherence to the basement membrane: Integrins Invasion of the peritoneal-blood barrier: MMP-1, MMP-2, MMP-7, MMP-9, MMP-13, MMP-14 ↑ TIMP-1, TIMP-2, TIMP-3, TIMP-4 uPA/uPAR Plasminogen activator inhibitor-1 and -2
Proliferation and angiogenesis	Proliferation: EGFR, EGF, TGFα IGF-1, IGF-binding Protein-3 Angiogenesis: HIF-1α, HIF-1β VEGF/VEGFR

Adapted from Lemoine et al. [9]; used with permission
E-cadherin epithelial-cadherin, *N-cadherin* neural-cadherin, *EMT* epithelial to mesenchyme transition, *PC* polycystin, *ICAM* intercellular adhesion molecule-1, *PECAM* platelet-endothelial cell adhesion molecule-1, *VCAM-1* vascular adhesion molecule-1, *TNF-α* tumor necrosis factor-α, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *IFN-γ* interferon-γ, *CD43* Sialophorin, *HGF* hepatocyte growth factor, *SF* scatter factor, *MMP* matrix metalloproteinases, *TIMP* tissue inhibitor metalloproteinases, *uPA* Urokinase plasminogen activator, *uPAR* Urokinase plasminogen activator receptor, *EGFR* epidermal growth factor receptor, *EGF* epidermal growth factor, *TGFα* tumor growth factor α, *IGF-1* insulin like growth factor-1, *HIF* hypoxia inducible factor, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor

Molecular and Genetic Considerations in HIPEC

It is generally acknowledged that the synergism of hyperthermia and IP chemotherapy may be in part due to increased cell permeability and improved membrane transport [1]. However, surprisingly little is known about the impact of HIPEC on the molecular and genetic level. Such information could serve highly valuable to developing targeted treatment strategies. The putative effect of HIPEC is often extrapolated from the

Table 7.2 Cellular effects of hyperthermia

Destabilization of the cell membrane
Changes in cell shape
Impaired transmembrane transport
Changes in membrane potential
Modulation of transmembrane efflux pumps
Induction of apoptosis
Impairment of protein synthesis
Protein denaturation
Aggregation of proteins at the nuclear matrix
Induction of heat sensitive protein synthesis
Impairment of DNA and RNA synthesis
Inhibition of enzyme repair
Altered DNA conformation
Alteration of gene expression and signal transduction
Inhibition of oxidative metabolism

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effect of hyperthermia in inhibiting angiogenesis, inducing apoptosis, denaturing cell membrane protein denaturation, and interfering with DNA repair [15]. Table 7.2 summarizes some of the cellular effects of hyperthermia [16]. This portion of the chapter will focus on data derived from combined hyperthermia and IP chemotherapy.

Histologic Alterations

The Pittsburgh group examined histologic alterations in peritoneal tumor and nonneoplastic peritoneal tissue samples from patients undergoing CRS/HIPEC for carcinomatosis due to appendiceal or colorectal cancer. Conventional histologic analysis demonstrated extensive subendothelial inflammatory infiltrate, endothelial activation, mesothelial karyolysis, and fibrin surface deposition following HIPEC. Immunohistochemical markers for early DNA damage (mesothelial nuclear γH2AX) and early necrosis (high-mobility group box 1 (HMGB1)) were found to be increased in CRS and HIPEC. H2AX is a component of histone octamer in nucleosomes; it is phosphorylated in response to breaks in double-stranded DNA, as an early step in recruiting DNA repair proteins. High-mobility group box 1 is a DNA-binding nuclear protein that may stimulate downstream inflammatory effects when released in the extracellular environment, and its presence may be an indicator of early necrosis [17]. Pelz et al. studied the effects of HIPEC with Mitomycin C in a rat model of colon carcinomatosis. Tumor cells demonstrated clear shrinkage and partial loss of contact, presence of thromboses of larger adjacent vessel on the tumor-muscle border, and macrophage infiltration. All of these findings were considered indicators of irreversible cell damage [18].

Assessment of Tumor Burden after HIPEC

Intraperitoneal free cells (IFCC) may result from spontaneous exfoliation of cancer cells from the primary tumor or from iatrogenic dissemination during CRS. Ji et al. studied the effect of HIPEC on IFCCs by examining carcinoembryonic antigen (CEA) and cytokeratin-20 (CK20) mRNA with conventional and real-time quantitative RT-PCR in the peritoneal fluid of 50 patients undergoing CRS/HIPEC for gastric, colorectal, epithelial ovarian, or appendiceal cancer. Positive cytology rate was 22% post-HIPEC, compared to 100% pre-HIPEC. The pre- and post-HIPEC rates of CEA and CK20 mRNA detection by conventional RT-PCR were 100% vs 86% (p-value = 0.012) and 100% vs 96% (p-value = 0.495), respectively. However, by quantitative RT-PCR, relative expression of CEA (36% of patients) and CK20 mRNA (34% of patients) was both significantly decreased post-HIPEC. In this study, the authors concluded that not only can HIPEC eradicate IFCCs, but it may also result in partial cytologic cure [19]. Though the mechanisms of action of HIPEC are unclear, they may include tumor microvessel embolization at the tissue level, perturbations of cell homeostasis and energy metabolism, and disruption in cell membrane integrity [20]. Baratti et al. investigated the prognostic value of tumor markers in patients with pseudomyxoma peritonei (PMP). Baseline and serial CEA, CA 19–9, CA-125, and CA 15.3 were obtained in CRS/HIPEC patients. Normal CA-125 correlated with the likelihood to achieve a complete cytoreduc-

tion, which in turn is a prognostic factor in PMP. Baseline elevated CA 19–9 was an independent factor of worse progression-free survival after CRS/HIPEC [21]. The Pittsburgh group obtained baseline CEA, CA 19–9, and CA-125 prior to CRS/HIPEC. At least one tumor marker was elevated in 70% of patients prior to CRS/HIPEC, allowing for surveillance. CA 19–9 was found to be a marker for progression, and CA-125 was associated with shorter survival [22].

Gene Expression

MicroRNAs (miRNAs) are small noncoding RNA sequences containing about 22 nucleotides that function in RNA silencing and posttranslational regulation of gene expression. Up- or downregulation of specific miRNAs has been associated with cancer development. Zhang et al. demonstrated that microRNA-218 (miR-218) was upregulated by greater than eightfold in the serum of patients with advanced gastric cancer after undergoing CRS/HIPEC. In addition, miR-218 increased chemosensitivity to cisplatin in vitro and in vivo by inducing apoptosis [23]. Long noncoding RNAs (lncRNAs), defined as transcripts longer than 200 nucleotides, have also been shown to be involved in the cancer development and progression. Zeng et al. identified two important lncRNAs, BC031243 and RP11–356I2.2, in the serum of patients with gastric cancer that were differentially expressed before and after CRS/HIPEC [24]. Further investigation is required to understand the biologic significance of these small molecules and the utility of targeting them to prevent cancer progression.

DNA Damage Response to HIPEC

There is a large body of literature suggesting that hyperthermia increases cell sensitivity to DNA damaging agents (such as cytotoxic chemotherapeutic agents) as well as a number of studies indicating a direct effect of hyperthermia on DNA damage. The latter is more difficult to unravel as there are profound differences in studies examining mild hyperthermia (41–43 °C), as utilized during HIPEC, versus more severe hyperthermia (>43 °C). The most sophisticated recent studies reveal that hyperthermia appears to act to inhibit mechanisms of DNA repair, and in this manner may act synergistically with cytotoxic agents. For instance, several studies have demonstrated that mild hyperthermia inhibits DNA repair of homologous recombination occurring after double strand breaks induced upon DNA damage. Repair occurs during the S-phase and G2-phase of the cell cycle via a cascade requiring the RPA, RAD51, and the BRCA2 proteins. Hyperthermia above 40 °C was found to inhibit the accumulation of RAD51 at sites of DNA damage by targeting BRCA2 for proteasomal degradation. Schaaf et al. studied the effects of hyperthermia in combination with chemotherapy and noted that hyperthermia delayed the repair of DNA damage caused by cisplatin or doxorubicin, by acting upstream of multiple repair pathways to block histone polyADP-ribosylation. This histone modification which is required for DNA repair is similarly targeted by PARP inhibitors. Not surprisingly, the investigators found that hyperthermia and PARP inhibitors had similar effects on cell cytotoxicity and impact on DNA repair function in models of ovarian and colon cancer. Importantly, these studies were performed in BRCA-competent cells, which comprise the majority of cancers that give rise to peritoneal metastases treated by CRS/HIPEC [25]. Finally, a recent study demonstrated that 42 °C of hyperthermia induced degradation of BRCA2 in cell lines and in human tumors treated ex vivo, also suggesting the potential for therapeutic synergism of hyperthermia and PARP inhibition [26]. These studies raise provocative questions regarding both the potential for enhancing the efficacy of CRS/HIPEC via selection of specific chemotherapeutic agents and for their combination with DNA damage repair inhibitors.

Heat Shock Protein Expression

Heat shock proteins (HSP) act as molecular chaperones inside cells and are protective against

cellular stressors, such as ischemia, heat stress, and oxidative stress. A study by Pelz et al. established an in vitro model of hyperthermia utilizing the HT-29 colon carcinoma cell line treated with HIPEC between 39 °C and 43 °C. Upregulation of HSP27, HSP72, and HSP90 mRNA was found at 41 °C and 43 °C. Increased protein expression of HSP70/72 by Western blot analysis was demonstrated at 30 minutes after exposure to HIPEC, while increased protein expression of HSP27 and HSP70/72 was seen at 12 hours. Tumor samples from patients undergoing CRS/HIPEC for a variety of histopathologic subtypes (appendiceal cancer, diffuse malignant mesothelioma, gastric cancer, ovarian cancer, pancreas cancer, and appendiceal carcinoid) were analyzed for HSP gene expression. Upregulation of HSP70/72 and HSP90 mRNA was found at varying levels on quantitative RT-PCR. This study postulates that targeting HSP in HIPEC procedures may be a promising therapeutic strategy [27]. Tu et al. subjected SGC7901 gastric cancer cells to HIPEC and found mRNA and protein expression of the HSP70 and HSP90 to be elevated. Serum levels of HSP70 and HSP90 were collected from patients undergoing CRS/HIPEC for gastric cancer. The serum concentration peaked at 12 hours and 18 hours post-HIPEC, respectively, and returned to normal levels at 24 hours. The authors advocated a second round of HIPEC at least 24 hours following the initial treatment in order to minimize any potential thermoresistance or chemoresistance of tumor cells [28]. As several HSP inhibitors are now reaching early Phase clinical trials, it will be of great interest to study their activity in the context of CRS/HIPEC.

Danger-Associated Molecular Patterns

Danger-associated molecular pattern (DAMP) molecules are endogenous molecules that are released upon tissue damage. They may elicit a systemic inflammatory response and induce an immunosuppressive state, leading to increased susceptibility to nosocomial infection. A study by Leitje et al. collected blood samples of 20 patients undergoing CRS/HIPEC at various time-points. Circulating levels of DAMP (Table 7.3) and cytokines [TNF-α, IL-6, IL-8, IL-10, macrophage inflammatory protein (MIP)-1α, MIP-1β, MCP-1] were measured and were all found to be increased significantly following CRS/HIPEC. Increase in HMGB-1 correlated with a decrease in HLA-DR expression, which may increase vulnerability to sepsis due to the impairment of optimal presentation of microbial antigens to T-cells [29]. Peak HMGB-1 concentrations were found to be significantly higher in the subset of five patients who went on to develop wound infections [30]. The implications are that release of DAMPs post-HIPEC could impair immune responses that result in clearing of tumor cells. Studies exploring this hypothesis and the potential therapeutic value of targeting DAMPs are clearly of interest.

Table 7.3 Danger-associated molecular pattern (DAMPs) [30]

Danger-associated molecular pattern (DAMPs)
Heat-shock proteins (HSP70)
HMGB-1
S100 proteins (S100A12, S100A8/S100A9)
Nuclear DNA
Mitochondrial DNA
Lactate dehydrogenase

Somatic Mutations as Prognostic Factors Post-CRS/HIPEC

As next generation sequencing has become widely available, several studies have characterized somatic mutations within rare peritoneal surface malignancies as a means to understand their biology, and in the hopes of revealing actionable alterations. Several studies have examined this data in the context of patient prognosis. Singhi et al. analyzed the prognostic implications of mutations in 86 patients with malignant peritoneal mesothelioma. They noted that loss of expression of the tumor suppressors CDKN2A and NF2 were each prognostic of poor survival. Furthermore, loss of function of both genes (by mutation or epigenetic silencing) resulted in a hazard ratio for death of 4.4, which was more potent than even the peritoneal cancer index or the extent of cytoreduction [31]. The most com-

mon mutational event in peritoneal mesothelioma is in the BAP1 gene. Germline mutation in BAP1 is associated with increased risk for both pleural and peritoneal mesothelioma. Interestingly, a study by Baumann et al. demonstrated that 23 mesothelioma patients with inherited BAP1 mutations had a favorable prognosis compared to mesothelioma patient survival as recorded in the Surveillance, Epidemiology, and End Results (SEER) database [32].

Loss of expression of the tumor suppressor SMAD4 was shown by Davison et al. to be associated with high tumor grade and a poor prognosis in mucinous neoplasms of the appendix, the majority of which were treated with CRS/HIPEC [33]. Mutations in the GNAS gene are among the most common in mucinous appendiceal tumors. The effect of GNAS mutations on prognosis remains unclear as studies have demonstrated somewhat conflicting findings. Alakus et al. characterized mutations in peritoneal metastases from low- and high-grade mucinous appendiceal neoplasms and found GNAS to be more common in low-grade tumors [34]. In contrast, Singhi et al. found GNAS mutations to be prevalent in both low- and high-grade tumors but to hold no prognostic significance [31]. A more recent study of patients with recurrent pseudomyxoma peritonei treated with capecitabine and bevacizumab found that GNAS mutations were predictive of poorer survival. Finally, Ang et al., in a study of appendiceal tumor subtypes, noted that low-grade tumors were enriched for GNAS mutations, whereas high-grade tumors were enriched for p53 mutations. Interestingly, the coexistence of GNAS and p53 mutations conferred a more favorable prognosis than p53 mutation alone [35]. Clearly, additional studies are required to further our understanding of the prognostic impact of gene mutations in peritoneal surface malignancies and how they may interact with response to CRS/HIPEC and systemic therapies.

Summary

Combined hyperthermia and intraperitoneal chemotherapy have been demonstrated in many in vivo and in vitro studies to produce a synergistic antitumor effect. Some of this effect has been attributed to the direct cytotoxic effects of the chemotherapeutic agent, which is essentially governed by pharmacokinetics. However, HIPEC has been shown to produce histologic alterations and cellular stress on the molecular level. Selective gene expression may occur in response to cellular stress, which may provide potential targets for therapy or may provide prognostic information about morbidity or survival. The molecular and genetic effects of HIPEC are extremely complex and require further study to fully elucidate their impact.

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