Cancer of Other Origin

12

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Introduction

The previous chapters in this section discussed our current knowledge of the biology and clinical relevance of lung, ovarian, and breast carcinoma metastasis in serous effusions, with similar analysis of the native cancer of the serosal cavities, malignant mesothelioma. As discussed in Chap. 7, a vast array of malignant tumors may additionally be diagnosed in serous effusions. The majority of these cancers are highly aggressive, and their detection in effusion specimens precludes any curative approach, underscoring the need to better characterize them with respect to the presence of potential molecular targets. However, the rarity of the majority of these entities has undoubtedly contributed to the scarceness of research aimed at better understanding their biology and improving therapy. The only obvious exception is cancer originating in the gastrointestinal (GI) tract, which has been the subject of a relatively large number of publications. This chapter will consequently focus on these tumors, followed by a brief discussion of the few published investigations of malignant melanoma and sarcomas in effusions. New diagnostic markers that are useful in the diagnosis of these cancers by immunohistochemistry are discussed in Chap. 7.

GI Cancers

The sites of origin for gastrointestinal cancers disseminating to effusions are the stomach, pancreas, liver, biliary tract, colorectum, and esophagus. With the exception of colorectal carcinoma, all these organs give rise to highly aggressive tumors, which are associated with very poor 5-year survival, often in the range of 5–10% [1–9]. Not unexpectedly, the detection of carcinoma cells from these organs in effusions marks a subset of patients with a still worse outcome within this patient group [10–22]. The epidemiology of these malignancies and their clinical features are briefly discussed in Chap. 7.

While cancers originating from the GI tract are biologically different, two or more types have been analyzed together in several studies, making it logical to discuss these tumors as one group, focusing on a single site of origin where relevant. Generally, studies of GI tract cancers have focused on three issues:

1. Improving the diagnosis, especially in the differential diagnosis from benign effusions in conditions mimicking cancer (e.g., cirrhosis).
2. Understanding aspects of tumor biology.
3. Assessment of new therapeutic modalities.

Diagnostic Approaches

Despite the central role of immunohistochemistry in effusion cytology, several other approaches have been evaluated in this context as an adjunct to morphology.

Cascinu et al. analyzed the levels of soluble carcinoembryonic antigen (CEA), CA 19.9, CA 15.3, CA 125, mucin-like carcinoma-associated antigen (MCA), α-fetoprotein (AFP), and prostate-specific antigen (PSA) in 89 effusion supernatants, including 30 gastric, 11 colorectal, and 6 liver carcinomas, as well as 5 prostate carcinomas, using an immunoradiometric assay. CEA, CA 19.9, and CA 125 levels were above cutoff levels in all colorectal and in the majority of gastric carcinomas. AFP and PSA identified all liver and prostate carcinomas, respectively, with high degree of specificity [23].

Yu and co-workers analyzed 112 effusions, consisting of malignant effusions, the majority of which were lung carcinomas, benign exudates, and cytology-negative effusions from cancer patients for mRNA levels of *MUC1*, *MUC2*, and *MUC5AC*. The malignant effusion group included four gas-

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tric carcinomas, whereas the cytology-negative group included five specimens from patients with liver carcinoma and one from pancreatic carcinoma. *MUC1* and *MUC5AC* levels were significantly higher in malignant compared to benign specimens. They were additionally higher in the cytology-negative group compared to benign effusions, and in the former group, 11/23 specimens were subsequently found to contain tumor cells which were not detected in the initial morphological examination [24].

An additional study investigated the presence of *KRAS* mutations in 34 malignant and 15 benign cytological specimens, including 41 effusion supernatants, using single-strand conformation polymorphism (SSCP) analysis. The majority of malignant specimens were from patients with GI cancers. *KRAS* mutations were found in 8/9 pancreatic carcinomas, as well as in 2 colorectal and 1 gastric carcinoma, and findings were similar in analysis of effusion cell pellets and solid lesions. The assay identified three false-negative specimens, including two pancreatic and one colorectal carcinomas [25].

Telomerase, the enzyme that synthesizes telomeric DNA and contributes to the ability of cancer cells to avoid aging and replicate endlessly, has been the subject of a large number of diagnostic studies, including two which focused on GI cancer effusions.

Analysis of telomerase expression using the telomeric repeat amplification protocol (TRAP) assay was performed on 95 ascites specimens, including 40 HCC, 31 non-HCC GI carcinomas (10 gastric, 10 pancreatic, 8 colon, 3 cholangiocarcinomas), and 24 cirrhosis samples. The assay was positive in 16/31 (52%) and 10/40 (25%) of non-HCC GI carcinomas and HCC, compared to 1/24 (4%) of cirrhosis specimens, performing better than morphology in both malignant entities [26].

In an additional study, 25 malignant, including 14 GI carcinomas (9 HCC, 2 colon, 2 gastric, and 1 pancreatic carcinoma), and 47 benign specimens, the majority from patients with cirrhosis, were analyzed using the same assay. The TRAP assay was positive in 6/9 HCC and 4/5 of the non-HCC tumors, compared to 2/47 benign specimens, performing better than cytology also in this series [27].

The diagnostic role of Newcastle disease virus expressing the enhanced green fluorescent protein (NDV-GFP) was studied in gastric carcinoma washings. GFP-positive cells were found in 6/6 cases in which laparoscopy showed the presence of metastatic disease, compared to 3/6 specimens diagnosed by cytology [28].

The diagnostic value of flow cytometry (FCM) in the diagnosis of serous effusions based on the presence of specific leukocyte populations was assessed in several studies. Cornfield and Gheith compared the natural killer (NK) and T-cell populations in 30 benign and 30 malignant effusions, the latter including 5 GI carcinomas [29]. CD16+/CD56+ NK cell counts were significantly higher in malignant effusions, though only modestly ($p = 0.04$). Wang et al. found significantly higher numbers of CD14+/CD163+ tumor-infiltrating macrophages, considered tumor-promoting, in malignant compared to benign effusions [30]. Effector memory CD8+ T-cell levels were significantly higher in blood and pleural fluid from healthy controls compared to patients with malignant pleural effusion, pleural metastases, or benign asbestos-related lesions [31].

HCC deserves separate discussion in this context, as it expresses tumor markers shared by few other cancers, such as AFP, glypican-3, Hep-Par1, and arginase-1 [32]. In the last two decades, several diagnostic approaches were studied with the aim of differentiating HCC (or cancer in general) from cirrhosis.

The levels of α1-antitrypsin were reported to be higher in malignant ascites, including eight HCC specimens, compared to ascites from patients with cirrhosis, and this assay performed better than measurement of total protein ascitic concentration or the serum-ascites albumin gradient [33].

In an additional study of 149 ascites specimens, including 46 HCC, the concentrations of fibronectin, albumin, total protein, lactate dehydrogenase, and CEA were shown to be significantly higher in malignant non-HCC compared to benign specimens, whereas the opposite was true for the serum-ascites albumin gradient. However, none of these parameters differentiated chronic liver disease from HCC [34]. In contrast, fibronectin concentration was significantly higher in HCC ($n = 33$) compared to cirrhosis specimens ($n = 89$) in the series of Colli et al. [35].

Analysis of free fatty acid levels in 14 malignant (predominantly GI cancers, including HCC) and 19 cirrhotic ascites showed significantly higher levels in the former group. Free fatty acid and albumin levels were strongly interrelated [36]. Parenthetically, in situ hybridization for albumin mRNA using a digoxigenin-labeled oligonucleotide probe as complement to AFP immunohistochemistry was reported to be useful in HCC effusion cytology [37].

Miédougé and co-workers measured serum and ascites AFP in specimens from 125 patients, consisting of 31 HCC, 14 non-HCC cancers, and 80 benign cases. AFP serum levels were higher than ascites levels, but in both specimen types AFP, levels were significantly higher in HCC compared to the two other diagnostic categories. A diagnostic specificity of 95% was associated with a sensitivity of 67.7%, which was not improved by calculating the ratio between AFP and albumin or total protein [38].

Another marker suggested as useful in differentiating between HCC and cirrhosis is the nucleoside pseudouridine, product of RNA catabolism. In analysis of 54 cirrhosis and 17 HCC ascites specimens, this marker had a sensitivity of 88.2% and a specificity of 90.8% in diagnosing HCC [39].

The levels of vascular endothelial growth factor (VEGF) and the v6 isoform of the adhesion molecule CD44 (CD44v6) were significantly higher in malignant ascites (=23), including 14 GI carcinomas (6 gastric, 5 colonic, 2 HCC, 1 pancreatic) compared to cirrhotic (n = 26) or tuberculous (n = 8) ascites. There markers were consequently suggested as adjunct in this differential diagnosis [40]. Similar findings with respect to VEGF were reported in another study, in which 25 malignant ascites specimens, including 7 colon and 6 gastric carcinomas, were compared to 4 effusions from patients with cirrhosis [41].

Kraft and co-workers measured VEGF levels in 445 sera samples, including 212 samples from patients with cancer, among which 48 were of GI origin [42]. VEGF levels in sera from patients with GI or ovarian carcinomas were significantly higher compared to normal subjects. Analysis of 56 effusion specimens, including 9 from GI cancer patients, showed considerably higher VEGF levels in this material compared to matched serum samples.

Tumor Biology

The majority of studies in which biological and clinical aspects of GI cancers have been studied focused on gastric carcinoma. Many of the molecules discussed in this section have already been introduced in previous chapters, where their biological role is discussed.

Surface Molecules

Several studies analyzed the expression of cell surface molecules in gastric carcinoma. Tamai et al. analyzed 51 gastric carcinoma specimens for CEA expression by FCM. Tumor cell expression was unrelated to serum CEA levels or to patient survival, the latter available for 39 patients, although higher CEA expression was associated with shorter survival in the group of 8 patients with signet ring cell carcinoma [43]. In another study, serum and ascites CEA levels were measured in 119 patients with peritoneal carcinomatosis. Ascites CEA levels were higher than the corresponding serum levels, and higher levels in ascites were associated with shorter survival in univariate and multivariate analysis. In contrast, neither serum CEA nor the findings in cytological examination, in which 54.6% of specimens contained tumor cells, correlated with survival. Ascites CEA levels additionally correlated with treatment response in patients with serial measurements [44].

Analysis of the expression of the cell-cell adhesion molecule E-cadherin in 21 primary gastric carcinomas showed reduced or absent protein expression in poorly differentiated tumors with single-infiltrating cells compared to better differentiated ones. Tumor cells from 11 malignant effusions, including 7 gastric, 2 pancreatic, and 2 pulmonary carcinomas, were E-cadherin-negative in all but one specimen by immunofluorescence [45].

Gastric carcinoma cells in ascites specimens (n = 20) were shown to frequently express epidermal growth factor receptor (EGFR) and the CD44v9 isoform, with little expression of the v6 isoform, using FCM. The latter finding differed from both normal gastric mucosa and primary gastric carcinomas, suggesting altered expression of this adhesion molecule along tumor progression in this malignancy [46].

Kitayama and co-workers analyzed 506 ascites and peritoneal washing specimens from 333 patients, of whom 300 had gastric cancer and 33 had liver cirrhosis, for CD45 and CD326 (EpCAM) expression by FCM. High tumor-to-leukocyte ratio using these markers was significantly associated with poor survival [47].

Proteases

The expression and activity of proteases were analyzed in several studies, with focus on the matrix metalloproteinases (MMP) family (see Chap. 9). MMP-2 and MMP-9 expression was analyzed in the abovementioned material studied for VEGF expression [40] using zymography. Both enzymes were absent from cirrhotic or tuberculous specimens, whereas 20 and 18 of 23 malignant specimens were positive for MMP-2 and MMP-9, respectively [48]. Koyama reported on increased expression of MMP-2, MMP-7, MMP-9, membrane-type-1 MMP (MT1-MMP; MMP-14), and the MMP inhibitors TIMP-2 and TIMP-4 in both tumor cells and tumor-infiltrating lymphocytes from gastric carcinoma effusions (n = 20) compared to benign gastric mucosa (=20) and primary carcinomas (=15) using FCM [49]. A subsequent study by this author applying the same method documented the presence of these enzymes on α-smooth muscle actin-positive myofibroblasts from 20 gastric carcinoma effusions [50].

The Immune Response

As in other cancers, the interaction between the host immune response and tumor cells has been the subject of a relatively large number of studies of GI cancers, with focus on gastric cancer. As in other tumor systems, many of these studies provide evidence for altered or deficient immune response in this setting.

Expression of Fas ligand (FasL) by FCM was found in benign gastric mucosa and in gastric carcinoma cells, with highest levels in effusions, whereas tumor cells had little Fas

receptor (FasR) expression and little apoptosis. Tumor-infiltrating lymphocytes expressed both FasL and FasR and underwent apoptosis, suggesting that they may be attacked by carcinoma cells in the tumor environment [51]. Another study by the same group showed high expression of TRAIL and its receptors DR4, DR5, and DcR2 (see Chap. 9) on gastric carcinoma cells in primary carcinomas and effusions, with little apoptosis. Tumor-infiltrating CD3-positive T lymphocytes in effusions similarly expressed these molecules but underwent a greater degree of apoptosis [52]. The authors concluded that gastric carcinoma cells were resistant to Trail-mediated apoptosis, whereas lymphocytes were susceptible, probably through tumor-mediated attack on the immune system [52]. In a third paper by this author, expression of the apoptotic proteins caspase-3, caspase-8, and caspase-10, the anti-apoptotic proteins cFLIP and survivin, and the transcription factor NF-κB in tumor-infiltrating lymphocytes increased from benign mucosa through primary gastric carcinoma to malignant effusions. Tumor cells in primary and metastatic carcinomas had increased levels of cFLIP, survivin, and NF-κB, with highest level in carcinoma cells in effusions, suggesting their involvement in the inhibition of apoptosis in this cancer [53].

Analysis of 23 ascites specimens showed association between transforming growth factor-β1 (TGF-β1) mRNA expression in tumor cells and reduced activity of NK cells [54]. Of note, TGF-β1 serum levels were elevated in sera from patients with HCC, as well as in those with cirrhosis, compared to normal subjects, with similar findings for the TGF-β family member activin and its inhibitor follistatin. The levels of these molecules in 16 ascites specimens showed no relationship with those in matched sera [55].

Sasada et al. analyzed the presence of CD4 + CD25+ regulatory T cells, which have an immunosuppressive effect, in sera from 149 patients with GI malignancies. The proportion of this cell population was higher in samples from cancer patients compared to controls and was associated with shorter survival. CD4 + CD25+ regulatory T cells were additionally found in 7 ascites specimens that consisted of 6 gastric and 1 pancreatic carcinoma, with their percentage ranging from 30.3% to 75.9% of CD4+ T cells [56]. In agreement with these data, the percentage of CD4 + CD25 + $CD127^{low/-}$ regulatory T cells was higher in the blood of 57 gastric carcinoma patients compared with controls, and these cells were found in primary carcinomas, lymph nodes, and ascites from the studied patients [57].

Ormandy et al. found higher percentage of CD4 + CD25+ regulatory T cells in the blood of HCC patients compared with specimens from patients with cirrhosis, infection by the hepatitis viruses HBV and HCV, and healthy controls. Three analyzed ascites specimens had comparable presence of CD4 + CD25+ regulatory T cells. CD4 + CD25+ regulatory T cells were anergic toward T-cell stimulation and suppressed proliferation and cytokine production in co-cultured CD4 + CD25- T cells in vitro [58].

In two studies, modification of the immune response against GI cancer was used as potential therapeutic approach. Kono et al. isolated tumor-associated lymphocytes from the effusion specimens of 11 gastric and 3 colon stage IV carcinoma patients. Cells were co-stimulated in the presence of autologous tumor with IL-2 and returned to the patients' effusions. Upregulation of T-cell receptor CD3-associated signal transducing ζ (zeta) molecules, which are often lost along tumor progression, was seen in 2 of 14 patients, but was unrelated to the minor clinical response observed in 3 patients [59].

In another study, immunotherapy for malignant ascites in gastric carcinoma with the streptococcal preparation OK-432 resulted in eight positive and four negative responses. TNF-α production in vitro by cells isolated from ascites was significantly higher in responders compared to nonresponders, and this was associated with mRNA expression of the Toll-like receptor *TLR4* and the presence of a CD11c + TLR-4+ cell population [60].

Chemokines, a family of cytokines that are mainly produced by and affect the function of leukocytes, promote tumor cell survival and tumor progression in non-hematological cancer (see Chap. 9). The chemokine CXCL12 and its receptor CXCR4, which have been shown to form an autocrine pathway in other carcinomas (e.g., breast carcinoma), were studied for their biological role in gastric carcinoma. In vitro and in vivo experiments showed a role for CXCL12 and CXCR4 in migration, tumor growth, and ascites formation, with activation of ERK and AKT signaling. CXCL12 mRNA and protein were detected in mesothelial cells from human tissues, and high levels of CXCL12 were measured in 19 ascites specimens from patients with gastric carcinoma. Comparison of CXCR4 expression in primary carcinomas from stage IV patients who had peritoneal carcinomatosis with tumors that metastasized to other organs showed significantly higher CXCR4 expression in the former group, supporting the role of this pathway in peritoneal metastasis in gastric cancer [61].

Molecular/High-Throughput Analyses

Two early studies applying traditional cytogenetics have documented multiple chromosomal aberrations in gastric carcinoma effusions. Misawa et al. analyzed 6 peritoneal and 1 pleural effusions and observed changes in chromosome number and structure in 6 of 7 specimens, most frequently involving chromosomes 3, 5, 7, 13, and 17 [62]. Trigo studied 5 pleural effusions from gastric carcinoma patients and found frequent trisomy of chromosomes 1, 3, 16, and 19 and monosomy of chromosomes 5 and 21. Structural changes were most frequently found in chromosomes 1, 4, 5, 9, and 17 [63].

Zojer and co-workers studied the cytogenetic profile of 12 primary pancreatic carcinomas and 25 effusions (22 peritoneal, 3 pleural) from patients with metastatic pancreatic carcinoma using interphase fluorescence in situ hybridization (FISH). Cytological examination identified carcinoma cells in 12/25 effusions. Six of the primary carcinomas were hyperdiploid with no chromosomal imbalances, whereas imbalances, affecting mainly chromosome 8, were found in all effusions. Two of ten analyzed malignant effusions were found to have *MYC* mutations. FISH analysis identified aneuploid tumor cells in cytology-negative specimens [64].

Gene expression analysis was applied to compare the molecular profile of a gastric carcinoma cell line isolated from a primary carcinoma to that of five cell lines isolated from effusions. Upregulated genes in effusions included, among others, those encoding for proteins mediating the epithelial phenotype and adhesion (keratins 7, 8, and 14, CD44, integrin α3, occludin, desmoplakin), drug metabolism (aldehyde dehydrogenase, aldo-keto reductase family I), apoptosis (TGFβ-induced anti-apoptotic factor), and signaling (caveolin 3). Downregulated genes included those encoding death-associated protein (apoptosis), integrin β4 (adhesion), insulin growth factor binding protein-2 (IGFBP2, growth and metabolism), and $p27^{kip}$ and histone deacetylase 3 (signaling). The expression of three genes (*KRT7*, *ALDH*, and *IP3R*) was observed in clinical malignant effusions from gastric carcinoma patients and was absent in washings from patients with benign diseases [65].

Proteomics analysis of 3 ascites specimens from patients with pancreatic ductal adenocarcinoma identified 816 proteins, of which 493 were found in all 3 specimens. Little overlap with ovarian carcinoma ascites was seen. Twenty proteins were chosen as potential tumor biomarkers, including known cancer-associated proteins such as MMP-2, stathmin, osteopontin, and neural cell adhesion molecule-1 (NCAM1) [66].

Exosomes are 30–100 nm vesicles which contain cell-specific cargo, including various lipids, proteins, functional mRNA, microRNA (miRNA), and long noncoding RNA (see Chap. 9). Tokuhisa et al. analyzed the exosomal miRNA profiles of gastric cancer in 6 malignant ascites specimens, 24 peritoneal lavage samples, and culture supernatants of 2 gastric carcinoma cell lines, in the aim of identifying microRNAs related to peritoneal dissemination.

miR-1225-5p, miR-320c, miR-1202, miR-1207-5p, and miR-4270 were overexpressed in malignant ascites, lavage specimens from patients with serosa-invasive tumors, and the highly metastatic cell line OCUM-2MD3. PCR validation of the observed differences for miR-21, miR-320c, and miR-1225-5p confirmed the findings for miR-21 and miR-1225-5p [67].

The potential of next-generation sequencing (NGS) in defining the molecular profile of GI cancers in effusion specimens is beginning to gain research focus. Lim and co-workers compared normal gastric mucosa, the primary tumor (six diffuse-type and two intestinal-type adenocarcinomas), and malignant ascites from eight patients using whole-exome sequencing.

Analysis of base substitutions showed a mutational signature dominated by C-to-A substitutions in malignant ascites, whereas tumors from patients who received adjuvant chemotherapy had a high rate of C-to-T substitutions and hypermutation in malignant ascites. Recurrent mutations linked to carcinogenesis were observed in *COL4A6*, *INTS2*, and *PTPN13*. Mutations in druggable genes included those in *TEP1*, *PRKCD*, *BRAF*, *ERBB4*, *PIK3CA*, *HDAC9*, *FYN*, *FASN*, *BIRC2*, *FLT3*, *ROCK1*, *CD22*, and *PIK3C2B*, whereas mutations in metastasis-associated genes were observed in *TNFSF12*, *L1CAM*, *DIAPH3*, *ROCK1*, *TGFBR1*, *MYO9B*, *NR4A1*, and *RHOA*. Pathway analysis showed enrichment of mutations in the Rho-ROCK signaling pathway in malignant ascites [68].

Other Cancers

Little data is available regarding the biology of non-GI cancers in effusions, with the majority of studies focusing on malignant melanoma.

Savoia and co-workers studied the diagnostic role of an RT-PCR assay for tyrosinase mRNA in detecting melanoma in biological fluids. Analysis of 17 specimens, including 8 effusions, identified tyrosinase mRNA in 12 cases, whereas cytology and immunocytochemistry detected tumor cells in 7 specimens. The five patients with positive tyrosinase assay and negative cytology and immunocytochemistry had radiological evidence of tumor and died within 4 months. The assay was additionally more sensitive than measurement of tyrosinase in peripheral blood [69].

Pirker studied the cytogenetic profile of melanoma cells in 48 samples from 46 patients, including 5 effusion specimens, using comparative genomic hybridization [70]. The most common alterations observed were gains within chromosomes 20q, 7q, 7p, 20p, 6p, and 17q and losses in 9p, 10q, 6q, 10p, 4q, and 11q. Amplification of the telomerase reverse transcriptase gene (*hTERT*) on 5p15.33 and the telomerase RNA component gene (*hTERC*) on 3q26 were found in 22% and 12%, respectively, and the former was common in effusion specimens. Chromosomes or chromosomal regions containing telomerase-suppressing activities at 3p, 4p, 6p, and 10p were frequently underrepresented in melanomas.

Andre analyzed 11 malignant effusions, including 2 melanomas, for the presence of exosomes. Melanoma exosomes contained the tetraspanin family member CD81 and HLA class I and II molecules, as well as the melanoma antigen

Mart1. The possibility of immunizing patients against tumor antigens in exosomes was investigated [71].

Mutation of the *b2m* gene, encoding for a component of the HLA class I machinery, was identified in two cell lines from one melanoma patient, isolated from a lymph node metastasis and pleural effusion, resulting in loss of HLA class I antigen presentation and postulated by the authors to be a mechanism mediating resistance to immunotherapy [72].

Research focusing on metastases from sarcomas or small round blue cell tumors in effusions is to date limited to a few case reports documenting the establishment of cell lines from these tumors [73–77]. However, these reports provide an example of how such cell lines may be useful for studying chromosomal aberrations and other biological characteristics of these tumors, thereby providing a possibility to test potential therapy.

Targeted Therapy and Concluding Remarks

Patients diagnosed with malignant effusions have grim outlook, and prognosis is particularly poor for those who are diagnosed with one of the cancers discussed in this chapter, even when conventional therapy such as chemotherapy and radiotherapy is applied. Consequently, prolonging survival is critically dependent on the ability to offer more novel therapeutics.

Initial efforts in this direction in the context of malignant effusions included the use of catumaxomab, a trifunctional antibody that binds to EpCAM and CD3, in treating gastric cancer patients [78]. In an additional study, effusion specimens from patients with GI cancers were shown to be informative in identifying genes related to the metabolism of chemotherapy agents [79].

The feasibility of analyzing the expression of molecules relevant for targeted therapy in effusion specimens has been documented for HER2 [80, 81]. Recently, the ability to culture tumor cells from effusion specimens in the aim of testing novel therapeutics has been shown in several studies. Yoo et al. studied bile duct cancer specimens from 40 patients, of whom 20 had stage I–III and 20 had stage IV disease at diagnosis, using a NGS targeted sequencing kit including 381 genes. Ascites or pleural effusion was available in 24 cases. Fifteen mutations were found in primary tumor specimens, affecting *TP53*, *NRAS*, *KRAS*, *ERBB2*, and *PIK3CA*. Patient-derived cultures were successfully established from effusions in 22/24 cases [82].

Supporting the latter report, Golan et al. succeeded in establishing primary cultures from 93/101 ascites specimens obtained from 32 pancreatic carcinoma patients. Cultures were successfully assessed for invasion and migration and epithelial-mesenchymal transition (EMT) characteristics, as well as for *KRAS* status and chemotherapy sensitivity [83].

Similarly, Lee and co-workers successfully established tumor cell cultures from 130/176 cancerous effusions, predominantly ascites specimens, the majority from patients with GI-cancers. Genomic profiling was successful in 116 cases, yielding detection of 181 mutations in 50 genes using the Ion AmpliSeq Cancer Panel v2 platform [84].

Although reports focusing on targeted therapy are currently limited to case studies, there is growing awareness that this approach is the way forward in treating these cancers. The benefit of targeting VEGF or of dual targeting of HER2 and MET was recently documented in metastatic gastric carcinoma [85, 86]. *CDK4* amplification was identified in refractory rhabdomyosarcoma diagnosed in a 27-year-old man, in whom metastatic tumor analyzed included ascites and pleural effusion specimens, suggesting this molecule may be a target for patient-tailored therapy [87]. A *BRAF* V600 K mutation was detected in a pleural effusion from a 74-year-old male with primary melanoma of the scalp in a recent report [88]. These reports suggest that malignant effusions may gain more relevance in the management of patients with metastatic cancer already in the near future.

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