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The genomic profile of pancreatic adenocarcinoma and its relationship to metastatic disease

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1. Introduction

More than half of patients with pancreatic adenocarcinoma have metastatic disease at presentation (Mauro et al., 2014). Prognosis for these patients is poor, with a median survival of only a few months. The overall survival of patients with pancreatic adenocarcinoma is less than 5% (Mauro et al., 2014; Wadell et al., 2015; Vincent et al., 2011). Cigarette smoking is a significant preventable risk factor that exposes the patient to numerous carcinogens, notably nitrosamines, which have been linked with activating mutations in the *KRAS* gene (Duell, 2012). *KRAS* mutations are the most common oncogene mutations found in pancreatic adenocarcinoma, and can be seen in 80% to 90% of tumors (Mauro et al., 2014; Furukawa, 2009). *CDKN2A* is the most commonly affected tumor suppressor gene and loss is present in 80% to 95% of cases (Mauro et al., 2014; McCleary-Wheeler et al., 2012). *TP53* mutations are seen in 50% to 75% of pancreatic carcinomas, and *SMAD4* inactivation can be found in 50% to 90% of pancreatic carcinomas (Mauro et al., 2014; Furukawa, 2009). *KRAS* mutations occur early, followed by *CDKN2A* losses and then *TP53, BRCA2,* and *SMAD4* inactivation (Mauro et al., 2014).

5% to 10% of patients with pancreatic cancer have a family history of the disease (Klein, 2012). An increased risk of pancreatic cancer has been found in patients carrying germline mutations in *BRCA2*, *PALB2*, *CDKN2A*, *STK11*, and *PRSS1* genes (Vincent et al., 2011). 5% to 17% of families affected by familial pancreatic cancer are carriers of a mutant *BRCA2* gene (Vincent et al., 2011), which has been shown to increase the risk for development of pancreatic carcinoma by 3.5 to 10-fold (Mauro et al., 2014). Carrying a *CDKN2A* mutation with a history of familial atypical mole-multiple melanoma increases the risk of developing pancreatic carcinoma by 13 to 47-fold (Mauro et al., 2014; Hong et al., 2011).

In patients with metastatic disease, metastasis to the liver is most common, followed by metastasis to the lung (Mauro et al., 2014; Tothill et al., 2013). Understanding the progression of metastatic disease is essential to the development of screening and treatment methods that may enhance patient survival. Additionally, knowing the molecular profile of metastatic cancer in relation to the primary tumor may be of particular use in patients presenting with new metastatic disease of unknown primary. Our aim in this study was to investigate the genetic relationship of metastatic pancreatic adenocarcinoma to its primary counterpart using next-generation sequencing technology.

2. Materials and methods

This study was approved by the Dartmouth Committee for the Protection of Human Subjects. Twenty-nine patients diagnosed with metastatic pancreatic adenocarcinoma between 2004 and 2015 were included in the study. Specimens of primary tumor included samples taken from six Whipple procedures and twenty-three fine needle aspirates (Fig. 1). Metastatic sites included liver (twenty-one cases), lung (four cases), omentum (two cases), ovary and femoral head (one case each). One case had metastasis to both skin and lung. Seven of these cases were fine needle aspirates, and nine were biopsies or resections. An attending pathologist reviewed the H&E stained slides from each case, and selected blocks of primary and metastatic tumors.

Formalin-fixed, paraffin-embedded tissue blocks from these cases were obtained for sequencing. Of the twenty-nine cases, thirteen cases had either no tissue remaining in one of the paired blocks or tumor content below the required 10%, which is the assay's minimum requirement established during the validation (De Abreu et al., 2016). Eleven of these thirteen cases were fine needle aspirate specimens. DNA extraction was performed on both primary and metastatic tumor of the 16 remaining paired cases using the QIAmp DNA FFPE kit (Qiagen, Valencia, CA, USA). DNA quantification and DNA quality were assessed using the PicoGreen Quant-iT™ Pico-Green® dsDNA Assay Kit (Invitrogen, Paisley, UK) and KAPA hgDNA Quantification and QC Kit (KAPA Biosystems, Wilmington, MA, USA). Following

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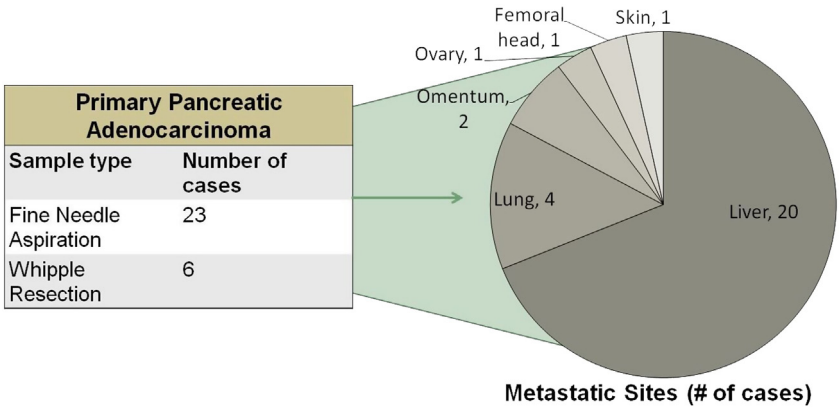

Fig. 1. Case selection. Paired primary and metastatic tumors were analyzed and compared using next-generation sequencing. 1 case had metastasis to both skin and lung.

library preparation, only 13 paired samples were sequenced on the 318 chips using the Ion Torrent PGM. Torrent Suite software (v4.0.2) was used for read mapping, alignment of sequences to human genome version 19 (hg19), variant calling, and coverage analysis. And Golden Helix SNP & Variation Suite (SVS) software (v8.2.1) was used for variant annotation and prediction of functional significance. After manual analysis using the Broad Institute's Integrated Genomics Viewer (IGV v2.3), paired genomic profiles of primary and metastatic tumor were compared. Then, public databases (such as ClinVar National Center for Biotechnology Information, dbSNP National Center for Biotechnology Information, and JAX-Clinical Knowledgebase – CKB) were used to obtain clinical importance of the variants.

3. Results

One liver biopsy, one fine needle aspirate of the pancreas and one fine needle aspirate of the liver failed qPCR, which represents one of the quality metrics established during validation (de Abreu et al., 2016). Of the remaining cases, ten contained liver metastasis, one contained both lung and skin metastasis, and two contained omental metastasis (Table 1). All cases contained *KRAS* variants in both primary and metastatic tumors. *KRAS* variants included G12V, seen in metastasis to liver (three cases) and omentum (two cases), G12D, seen in metastasis to liver (five cases), G12R, seen in metastasis to liver (two cases) and Q61H in metastasis to lung and skin. *TP53* variants were present in all but one case. *SMAD4* mutations were seen in omental and liver metastasis. *CDKN2A*, *FGFR1*, *ATM*, *FLT3* and *FBXW7* mutations occurred in one case each.

Two cases contained *APC* mutations in both primary and metastatic tumors. One case gained an *APC* E1317Q mutation in a metastatic liver lesion, the sole case demonstrating a difference in genomic profile between primary and metastatic lesions. All of the remaining cases retained the same genetic profile between primary and metastatic tumors (Fig. 2, Table 1).

4. Discussion

The majority of cases in our study retained their genomic profile between primary and metastatic tumors, with the exception of one case which gained an *APC* mutation in a metastasis to the liver. Considering the high prevalence of *CDKN2A* losses seen in primary pancreatic adenocarcinoma (Mauro et al., 2014; McCleary-Wheeler et al., 2012), it is interesting to note that only one of the thirteen cases in our study with metastatic disease contained a *CDKN2A* mutation. This study did not take into account the mode of metastasis (lymphatic or vascular) or other characteristic features of the tumor such as perineural invasion, which may also be interesting to explore. Investigating the molecular profile of tumors displaying perineural invasion in particular may be of interest considering its association with poorer prognosis (Ozaki et al., 1999). The application of targeted next-generation sequencing is increasingly being used in clinical laboratories for patient management, as well as for research purposes to further our understanding of primary and metastatic cancers (Tsongalis et al., 2014; Liu et al., 2014).

The option of utilizing molecular diagnostics in the workup of metastatic lesions can be proposed, considering that in this small study, all but one case retained its molecular profile. In this study, we were able to successfully perform next-generation sequencing on small cytology samples from fine-needle aspirates. Understanding the molecular profile of metastatic pancreatic cancer can potentially be useful in the diagnostic workup of metastatic tumors with unknown primary (Ozaki et al., 1999). Familiarity with the use of molecular technology as an adjunct tool in diagnosis has the potential not only to help establish the diagnosis of a lesion, but also for directing therapy as new discoveries are made that provide further insight into the pathophysiology of disease progression.

Patients with metastatic pancreatic adenocarcinoma are currently not considered candidates for surgical resection, and are typically treated with a Gemcitabine-based chemotherapy regimen (Mauro et al., 2014; Conroy et al., 2011), though survival remains dismal – typically lasting only a few months (Conroy et al., 2011). As of yet, targeted chemotherapeutic agents have shown poor results in improving the overall survival of patients with pancreatic adenocarcinoma (Goldstein et al., 2015). Recently, Alloway et al. reported the utility of patient-derived xenograft (PDX) models in furthering the understanding of targeted therapeutics in metastatic pancreatic cancer (Alloway et al., 2016). In their study, they observed that the therapeutic response of PDX models of pancreatic adenocarcinoma was similar in both primary tumor and patient-matched PDX models established from a metastatic site (Alloway et al., 2016). Further research of the genetics of metastasis will lead to a better understanding of the pathogenesis of pancreatic cancer and its impact on patient prognosis and response to targeted therapy (Alloway et al., 2016; Haeno et al., 2012). Larger studies may yield more information about the distribution of genomic variants in metastatic tumors.

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