HIGHLIGHT ARTICLE

KRAS in Pancreatic Cancer

Highlights from the "50th ASCO Annual Meeting". Chicago, IL, USA. May 30-June 3, 2014

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ABSTRACT

Pancreatic cancer is one of the most feared malignancies. The most common form of pancreatic cancer is adenocarcinoma arising from the ductal epithelium. KRAS is the most common oncogene that has been found to be mutated. However, targeting KRAS directly has been difficult. We do not know a lot about the relationship between KRAS and other signaling pathways. At the same time, little is known about the non KRAS mutated or wild type (WT) tumors. Most of the data that we have as far, as mutational status is concerned, has been obtained from the tumor itself and not from metastatic lesions. In this review, we discuss two abstracts (Abstracts # e15214 and # e15207) published in conjunction with the 2014 ASCO Annual Meeting. These discuss the relationship between KRAS and other signaling pathways and the differences between mutated KRAS and WT tumors. The studies found low rate of KRAS mutation in cells obtained from ascitic fluid. While the studies are small, these are novel findings that are worth exploring further. They increase our understanding of the biology of the disease and take us a step closer to treating this deadly malignancy.

Introduction

Pancreatic cancer is the fourth leading cause of cancer related deaths in the United States [1]. The majority of these tumors, 85% of them, arise from the ductal epithelium [2]. They have very poor prognosis with five-year survival rates reaching 6% [1]. Most patients present late and only about 15-20% of them can benefit from potentially curative surgery [3]. Gemcitabine is the cornerstone of treatment after surgery. It is also the main agent used alone or in combination with other agents in metastatic tumor [3]. Despite recent advancements in understanding of the biology of the disease, very little has changed as far as treatment is concerned. There is an urgent need for better therapy to target this challenging malignancy. In recent years, there has been an understanding that each patient's tumor is different and treatment needs to be individualized. We are slowing discovering the differences in tumors based on genetic patterns rather than histology alone.

What We Knew Before the 2014 ASCO Annual Meeting?

Pancreatic cancer arises from precancerous lesions. These include one microscopic lesion (pancreatic intraepithelial neoplasia) and two macroscopic lesions (intraductal papillary mucinous neoplasms and mucinous cystic neoplasm) [4]. Three broad categories of genes are involved in the pathogenesis of pancreatic adenocarcinoma. They include the oncogene KRAS, tumor suppressor genes such as TP53, p16/CDKN2A and SMAD4, and genes that encode DNA repair enzymes such ashMLH1 and MSH2 [5]. Of all of them, KRAS is the most prevalent and widely studied oncogene and has been found to be present in 90% of the tumors [2]. In normal inactive cells, KRAS is bound to GDP. In the presence of growth factors, the KRAS exchanges GDP for GTP. This is made possible by Ras guanine nucleotide exchange factor (RasGEF). Ras GTPase-activating proteins (RasGAPs0 return K-Ras to the inactive GDP-bound state by hydrolyzing GTP-GDP. Single amino acid substitutions at G12,13 or Q61 lead to the formation of mutated KRAS that are insensitive to GAP stimulation. This leads to accumulation of persistently GTP-bound and active KRAS which leads to pancreatic cancer formation. Several effector pathways are activated by the mutated KRAS. The most studied ones are the MAPK and P13 signaling pathway [6]. The details of these pathways are out of scope of this review. Figure 1 shows the sequence of events that lead to a persistently active KRAS and its defect on downstream effectors.

Inactive RAS bound to GDP is converted to active RAS in exchange for GTP. This is facilitated by Ras guanine nucleotide exchange factor (GEF). Active RAS is changed back to inactive RAS by Ras GTPase-activating proteins (GAP). A glycine to arginine mutation in codon 12 leads to persistently GTP bound KRAS. This mutated protein leads to downstream effector signaling via the MAPK (RAF, Mek1/2, ERK1/2) and PI3K pathways.

Key words Ascitic Fluid; Computational Biology; Genes, ras; Mutation; Pancreatic Neoplasms; Survival Analysis
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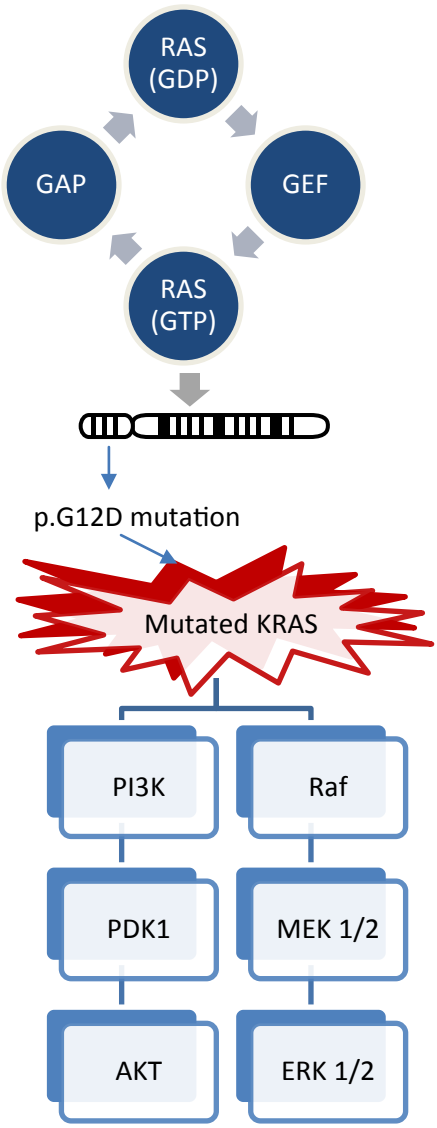

Figure 1. Mutant KRAS formation and its effector pathways.

What We Have Learned at the 2014 ASCO Annual Meeting?

This review article summarizes the recent work that was published in conjunction with the 2014 ASCO Annual Meeting. We discuss two abstracts which give us new input regarding KRAS mutations in pancreatic cancer.

RNA-seq and KRAS mutational status in ascitic pancreatic cancer cells: Novel results and distinct subsets (Abstract #e15214) [7]

This study is a retrospective analysis of patients with pancreatic adenocarcinoma who have developed ascites. The authors in a previous study have shown how to culture ascites derived pancreatic cancer cells [8]. They studied 19 such patients. They found that 17 out of the 19 ascites derived pancreatic cancer cells had wild type KRAS mutation while only 2 had mutated KRAS. The most common KRAS mutation occurs at codon 12 which leads to a single amino acid substitution form glycine to aspartic acid. However, the two KRAS mutations seen were glycine to arginine. Only 7 primary tumors were available for concordance studies. Out of them, 4 were found to be concordant (3 WT and one KRAS mutation from glycine to arginine). Out of the 3 discordant tumors, 2 had the common glycine to arginine mutation and one had glycine to valine mutation. This is a new discovering showing that mutated pancreatic cancer cells could be different from the original tumor. Figure 2 summaries these findings.

They also analyzed another 28 samples of pancreatic cancer cells obtained from ascitic fluid by RNA sequencing and performed unsupervised clustering on 31 transcriptosomes. They noted a few things: 1) Two clusters were formed, one with high KRAS expression and the other had low KRAS expression; 2) The high KRAS expression corresponded to low survival rates (8. 8 months) as compared to low KRAS expression (18. 2 months)(p=0. 0013); 3) As expected, high KRAS expression cluster was found to have more Ras/ Raf/ Mek, PI3 K and other signaling pathways while the low KRAS expression cluster had expression of proteoasome, oxidative phosphorylation and ribosome pathways. This suggests that the metastatic tumor cells might need to be targeted based on the KRAS expression status.

KRAS: To be or not to be targeted? Biologic and computational analyses in pancreatic adenocarcinoma (Abstract #e15207) [9]

This abstract describes 91 patients with pancreatic cancer who were retrospectively studied to look at the relationship between KRAS mutation and other signaling pathways at the expression level. The expression of 29 genes was studied. Out of the 91 patients, 49 had KRAS mutations while 42 had wild type (WT) KRAS genotype. This is different from other studies where 90% of patients have been described to have KRAS mutations. They found that the gene expressed in both these tumors were different. KRAS tumors had more expression of Sonic hedgehog (SHH) (p=0. 012) and Indian hedgehog (IHH) (p=0. 031).

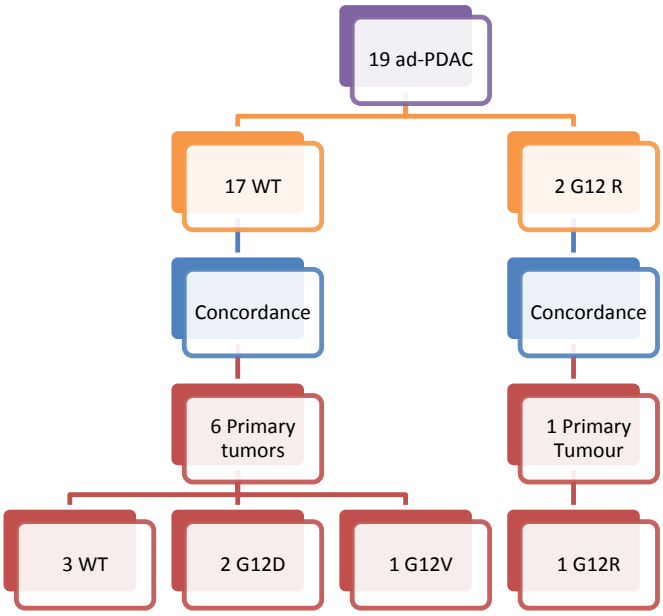

Figure 2. KRAS mutation and Concordance rates with primary tumor. Ad-PDAC: ascitic fluid derived pancreatic adenocarcinoma, WT: wild type, G12 D: glycine to arginine mutation, G12R: glycine to aspartic acid mutation, G12V: glycine to valine mutation.

WT KRAS had expression of Smad 4 (p=0. 03), Muc6 (p=0. 009), VEGFR-2 (p=0. 020) and VEGFB (p=0. 026). Based on these findings, they suggested that the WT tumor was biologically different and could benefit from angiogenic inhibitors. They also suggested that KRAS mutated tumors could benefit from Hedgehog inhibitors.

Bioinformatics analysis was done to see which of the KRAS mutations had more severe effect on protein expression due to pre-mRNA splicing, mRNA export and protein alteration. They found that G-R and G-S mutations had less severe alteration of protein expression. They conducted survival analysis between the different types of KRAS mutations and/or gene expression levels. They found no differences in survival between the different types of KRAS mutations as well between the KRAS mutated tumors and WT KRAS.

Discussion

The abstracts reviewed are hypothesis generating and have brought in valuable new information. For the first time, KRAS mutation status of pancreatic cancer cells in ascitic fluid has been described and has been found to be quite different than expected [8]. Both these papers report lower KRAS mutation status than previously described. The relationship between KRAS and other signaling pathway by gene expression sheds new light into the complex biology of the disease. This information could potentially be used to target pancreatic cancer differently.

In the first abstract (Abstract #e15214) [7], only19 patients with pancreatic cancer and ascites are described. This is a very small number and the finding that only a few patients had KRAS mutations could have well occurred by chance. We do not have any demographic or clinical data regarding these patients. Only 7 primary tumors were available for concordance studies. Larger studies are needed. The study also describes that patients in whom the pancreatic cancer cells obtained from the ascitic fluid had high KRAS mutations had low survival rates. This needs to be confirmed in larger studies. The metastatic tumor cells seen to be different in gene expression based on KRAS mutational status. Despite the above limitations, these findings are new and we need to now look at metastatic pancreatic cancer cells differently.

The second abstract (Abstract # e15207) [9] is a slightly larger study with 91 patients that studies the relationship between KRAS and other signaling pathways via gene expression. This study too is limited in size. Clinical and demographic data are not available as this time. Although 29 genes were studied, they could have overlooked other important genes. Their suggestion of using Hedgehog inhibitors in KRAS mutated tumors and angiogenesis inhibitors in WT tumors needs to be studied further. They found no differences in survival between the different KRAS mutations, as well as the WT tumors. These findings also give us new suggestions on targeting pancreatic cancer that need to be confirmed in larger studies.

KRAS mutation has long been the Holy Grail of pancreatic cancer. The gene is difficult to target directly and we need indirect ways of targeting it. Pancreatic cancer is a heterogeneous disease. As the information about the biology of the disease increases, we can target each patient's tumor differently. This is the era of individualized tumor therapy.

Conflict of Interest

The authors hereby state that there is no conflict of interest to disclose during the time of submission of this manuscript. We have no affiliation, financial agreement or other involvement of any author to disclose.

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