Cancer Letters ■■ (2015) ■■–■■

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Genomic profiling guides the choice of molecular targeted therapy of pancreatic cancer

Thomas S. Frank [a,1], Xiaotian Sun [a,b,c,1], Yuqing Zhang [d,1], Jingxuan Yang [a,b], William E. Fisher [e], Marie-Claude Gingras [e,f,*], Min Li [a,b,**]

[a] The Vivian L. Smith Department of Neurosurgery, The University of Texas Medical School at Houston, Houston, TX 77030, USA
[b] Department of Medicine, Department of Surgery, The University of Oklahoma Health Sciences Center, 975 NE 10th Street, BRC 1262A, Oklahoma City, OK 73104, USA
[c] Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
[d] Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[e] Michael E. DeBakey Department of Surgery, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[f] Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, N1519, Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 6 April 2015
Accepted 8 April 2015

Keywords:
Genomic profiling
Gene therapy
Mutations
Pancreatic cancer
Pharmacogenomics
SLC39A4 (ZIP4)

ABSTRACT

Pancreatic cancer has the worst five-year survival rate of all malignancies due to its aggressive progression and resistance to therapy. Current therapies are limited to gemcitabine-based chemotherapeutics, surgery, and radiation. The current trend toward "personalized genomic medicine" has the potential to improve the treatment options for pancreatic cancer. Gene identification and genetic alterations like single nucleotide polymorphisms and mutations will allow physicians to predict the efficacy and toxicity of drugs, which could help diagnose pancreatic cancer, guide neoadjuvant or adjuvant treatment, and evaluate patients' prognosis. This article reviews the multifaceted roles of genomics and pharmacogenomics in pancreatic cancer.

Introduction

The Human Genome Project (HGP) was initiated in 1990 with a project timeline of 15 years. The project was completed ahead of schedule in 2003, and identified over 3 billion base pairs and approximately 24,500 human genes [1]. However, a full human genome was not sequenced until 2007, with the use of Sanger sequencing technology [2]. One year later, scientists at the Human Genome Sequencing Center at the Baylor College of Medicine and 454 Life Sciences used a groundbreaking rapid-sequence 454 technology to sequence DNA pioneer Dr. James Watson's genome. At one hundredth of the cost of traditional capillary electrophoresis methods, this process took only two months to complete [3]. With new sequencing techniques and vast databases of information, the field of genomics has introduced revolutionary progress into standard practice, which is also defined as "personalized genomic medicine". Personalized genomic medicine uses genomic information to improve diagnoses and to guide the selection of molecular and gene therapy for each individual patient based on their specific genomic sequence.

Physicians now screen high-risk patients for genes that are linked to cancer, such as screening individuals at high risk of developing breast cancer for the *BRCA* gene [4]. Technology has progressed so quickly that direct-to-consumer DNA testing, in which gene chip analysis is performed on a saliva sample, is now a global industry [5]. The core components of this genetic innovation are single nucleotide polymorphisms (SNPs), which account for 90% of total DNA variations and are abundant, stable, and easy to identify. SNPs are observed in coding, noncoding, promoter, and enhancer regions of DNA sequences, and in microRNAs (miRNAs) and other non-coding RNAs. Moreover, SNPs in combination with immunohistochemistry may help identify the abnormal expression and function of proteins in human malignant diseases, especially pancreatic cancer with very poor outcome.

The National Cancer Institute (NCI) and Surveillance Epidemiology and End Results (SEER) showed that pancreatic cancer has a five-year survival rate of 5–6%. Since early detection is rare, most pancreatic cancer patients are diagnosed with advanced stages of tumors that are either unresectable or metastatic, with 27% and 53% having regional and distant metastases, respectively, at the time of diagnosis [6,7]. There have been no recent breakthroughs in pancreatic cancer treatment; gemcitabine-based therapy and surgery

* Corresponding author. Tel.: +1 713 798 1286; fax: +1 713 798 3741.
E-mail address: mgingras@bcm.edu (M.-C. Gingras).
** Corresponding author. Tel.: +1 405 271 1796; fax: +1 405 271 1766.
E-mail address: min-li@ouhsc.edu (M. Li).
[1] Frank, Sun, and Zhang contributed equally to this work.

http://dx.doi.org/10.1016/j.canlet.2015.04.009
0304-3835/

Please cite this article in press as: Thomas S. Frank, et al., Genomic profiling guides the choice of molecular targeted therapy of pancreatic cancer, Cancer Letters (2015), doi: 10.1016/j.canlet.2015.04.009

have been the standard of care for over a decade [7]. Chemotherapy options remain limited to therapies containing gemcitabine as a core component, either as part of a drug cocktail or as a therapeutic neoadjuvant or adjuvant to surgery [8]. This review focuses on the application of introducing personalized genomic medicine into the management of pancreatic cancer.

Pancreatic cancer is one of the most heterogeneous of all malignancies [9]. Genetic hallmarks of the disease include global genomic instability, referring to mutation, translocation, and insertion/deletion, and aneuploidy. Global genomic analysis has revealed twelve core signaling pathways which have genetic variations. The most common genetic alterations harbored in pancreatic cancer are within the KRAS, TGF-β, apoptosis, and cell cycle pathways, besides DNA replication and axon guidance [10,11]. Similar genetic variations in several inherited genetic disorders, like Lynch syndrome caused by the DNA mismatch repair (MMR) mutations, and hereditary breast–ovarian cancer caused by the *BRCA* mutations, also account for 5–10% of pancreatic cancer, especially familial pancreatic cancer [12–14].

The pancreatic tumor's genetic profile may allow physicians to determine: (1) tumor response to chemotherapy, radiation, or surgery, (2) "tailored" therapeutics, such as neoadjuvant, adjuvant, and gene therapy, and (3) efficient drug delivery approaches. This information is clinically important for enhancing treatment efficacy, lowering cytotoxicity, and improving the patient's quality of life. Developed resistance to multiple drugs is common in pancreatic cancer, the treatment of which could be optimized by obtaining useful information from genomic profiling. Besides, genetic profiling can also be used to predict prognosis, consequently preventing patients from undergoing a burdensome treatment that might not significantly prolong their survival.

Treatment optimization can be conducted using samples derived from surgical biopsy, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA), or circulating tumor cells (CTCs). Surgical resection of pancreatic tissues is still the current gold standard for biopsy, although less invasive methods, like EUS-FNA, are emerging. However, FNA-extracted cells may be difficult to distinguish malignant lesions from benign pancreatic diseases like chronic pancreatitis with similar morphological characteristics to pancreatic cancer. CTCs, as potential biomarkers for pancreatic cancer, are tumor cells from primary or metastatic sites that can be isolated from the peripheral blood, which can be also implemented as a "real-time biopsy" [15]. Recently, the deep sequencing potential is tested for the detection of *KRAS* mutation in serum. Yu et al. established a convenient and accurate method to screen plasma *KRAS* mutations with a sensitivity of 87.5% and an accuracy of 92.9%, which may be an especially useful alternative for diagnosis when tumor specimens are unavailable [16]. Thus, novel molecular and genetic biomarkers for pancreatic cancer are always in great need to improve accurate and early diagnosis.

Genomics of pancreatic cancer

The progression of pancreatic cancer and its genetic changes has been well recognized. In 2008, the results of whole-exome sequencing of 24 patients revealed an average of 63 genomic alterations, most of which were point mutations. *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* genes are the most frequently mutated [10]. In 2008, Biankin et al. conducted genomic sequencing in 142 patients with preoperative clinical stages I and II, and identified a total of 16 significantly mutated genes, including genes such as *ATM* and *MLL3* [11]. Other studies reported genes playing important roles are *BRCA1* [17], pancreatic and duodenal homeobox 1 (*PDX-1*) [16,18], and *SLC39A4* encoding ZIP4 [19–21]. We added *SLC39A4* to this high-yield list after discovering the significant correlation between its expression level and pancreatic cancer progression [19]. Key genes in pancreatic cancer are summarized in Table 1.

The *KRAS* mutations are present in over 90% of invasive pancreatic cancer, and are responsible for the progression from pancreatic intraepithelial neoplasms (PanINs) to pancreatic cancer [22,23]. *KRAS* is a proto-oncogene that, once point mutated and consequently activated, can recruit and activate growth factors and receptor signals for malignant transformation. Epidermal growth factor receptor (EGFR), which promotes the growth of tumor cells, is a direct upstream gene of KRAS [35]. In colon cancer, drugs targeting EGFR will lose efficiency if KRAS is activated. Thus, genetic screening before the administration of anti-EGFR therapy is necessary [36]. Another potential application of KRAS is using a stool sample and real-time methylation-specific polymerase chain reaction (MSP) as a detection method for pancreatic cancer [37]. In addition, plasma DNA sequencing may provide new insights into understanding carcinogenesis and making an early diagnosis [38,39]. EUS-FNA could permit physicians to biopsy pancreatic masses in order to further sequence *KRAS* [40,41].

During the progression from PanINs to pancreatic cancer, the tumor suppressor p16 is downregulated due to the loss of *CDKN2A* gene. Loss of function in p16 occurs early in 86–95% of sporadic pancreatic cancers [23]. Immunohistochemistry staining showed a significant correlation between lymphatic invasion and a lack of p16, exemplifying how this gene could be used to assess the staging of pancreatic cancer [42]. p16 as a tumor suppressor inhibits the cell proliferation by mediating the cell cycle. It has been shown that mutant p16 could participate in the development and progression of multiple human cancers [43–45]. p53 is another example that is commonly

Table 1
Genomics of pancreatic cancer.

Genes	Function	Significance in pancreatic cancer
KRAS	Proto-oncogene that recruits growth factors [22].	Present in 90% of invasive pancreatic cancer specimens [22].
CDKN2A	Regulates cell cycle [23].	Loss of function in 86–95% of patients with pancreatic cancer [23].
TP53	Arrests cell cycle, activates DNA repair, and initiates apoptosis [10].	Most frequently mutated gene in all cancers. No correlation found with survival [10].
SMAD4	Tumor suppressor gene that regulates growth of epithelial cells and extracellular matrix, plus TGF-B mediated cell growth [23].	22% of local pancreatic cancer with no metastases showed a loss of *DPC4*, compared with 75% of those with metastatic disease [24,25].
BRCA1/2	Involved in the repair of DNA double-stranded breaks [26].	*BRCA1/2* deficient cell lines were hypersensitive to PARP inhibitors [26].
PDX1	Responsible for embryonic development of the pancreas and present in mature beta cells [27,28].	Found at the infiltrate's leading edge and lymph node metastases, associated with TNM grading, cell proliferation [29] and reduced survival [30,31].
ATM	Goes in pairs and in the same way as *TP53* [32].	Significantly mutated in pancreatic cancer [33].
SLC39A4	Zinc importer [34].	Overexpressed in pancreatic cancer, and associated with increased aggressiveness and tumor growth [19].
Others (*MLL3*, *SLC16A4*, etc)	Associated with chromatin modification, DNA damage repair and other mechanism [11].	Defined as most significantly mutated genes by exome sequencing and copy number analysis in pancreatic cancer [11].

The key genes, their functions and the associations with pancreatic cancer are listed. The common genetic variations in those genes are also included.

Please cite this article in press as: Thomas S. Frank, et al., Genomic profiling guides the choice of molecular targeted therapy of pancreatic cancer, Cancer Letters (2015), doi: 10.1016/j.canlet.2015.04.009