Gene 679 (2018) 328–334

Contents lists available at ScienceDirect

Gene

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

Review

Prognostic value of circulating cell-free DNA in patients with pancreatic cancer: A systemic review and meta-analysis

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ARTICLE INFO

Keywords:
Circulating cell-free DNA
Prognosis
Pancreatic cancer
Meta-analysis

ABSTRACT

Because of the deep research about tumorigenesis mechanism, the cognition of cancer has been transferred to molecular level from morphology. Previous articles reported a potential connection between circulating cell-free DNA (cfDNA) and prognosis of pancreatic cancer. A total of 18 related articles including 1243 patients were enrolled to access the relationship between cfDNA and prognosis of pancreatic cancer. The hazard ratio (HR) was used to combine the univariate and multivariate results of included studies. Our result performed that the cfDNA had significant prognostic value in predicting OS (HR = 2.41, 95%CI: 1.93–3.02, I^2 = 60%) and PFS (HR = 2.47, 95%CI: 1.80–3.40, I^2 = 0%) in univariate analysis. The multivariate analyses about OS (HR = 2.57, 95%CI: 1.95–3.38, I^2 = 66%) and PFS (HR = 2.31, 95%CI: 1.47–3.64, I^2 = 0%) also showed significance. In conclusion, the cfDNA was a significant prognostic factor for OS and PFS in patients with pancreatic cancer. The mutation (Kras, ERBB2-exon17 and KrasG12V), circulating tumor DNA (ctDNA) presence, hypermethylation and higher concentration of cfDNA were both associated with worse survival results in pancreatic cancer.

1. Introduction

As a kind of malignant tumor from digestive tract, pancreatic cancer is the seventh highest cause of cancer-related death worldwide (World Health Organization, 2014), which resulted in 411, 600 deaths in 2015 (GBD 2015 Mortality and Causes of Death, Collaborators, 2016). It is difficult to diagnose and treat patients with pancreatic cancer, thus pancreatic cancer typically has a very poor prognosis with one year survival rate of 25% and five year survival rate of 5% (World Health Organization, 2014; American Cancer Society, 2010). In early localized and small tumors (< 2 cm) without lymph node metastases and extension beyond the capsule of the pancreas, complete surgical resection was associated with a low actuarial five-year survival rate of 18% to 24% (*Pancreatic Cancer Treatment Health Professional Version*, 2014). The diagnostic methods for pancreatic cancer such as computed tomography, endoscopic retrograde cholangio-pancreatography and endoscopic ultrasonography are frequently used nowadays, which increase diagnostic rate to a certain degree. For instance, endoscopic ultrasonography is able to differentiate pancreatic cancer with high sensitivity of 95% and specificity of 92% (Dabizzi et al., 2011; Hanada et al., 2015; Yang et al., 2014). What's more, surgical operation, chemotherapy and radiotherapy have reduced the risk of cancer progression and improved overall survival rate of pancreatic cancer patients. For example, complete resection (R0) prolonged survival time of 18–27 months compared with non-resectable pancreatic ductal adenocarcinoma (4–8 months) (Krška et al., 2015). However, despite the development of diagnostic and therapeutic methods, the survival rate of patients with pancreatic cancer still stays in a low level compared to other malignant tumor. Therefore, effective prognostic biomarker is of great importance to predict survival outcome, optimize treatment and monitoring strategies for high-risk pancreatic cancer patients.

Because of the deep research about tumorigenesis mechanism, the cognition of cancer has been transferred to molecular level from morphology. The tumor markers such as carbohydrate antigen 19-9 (CA19-

Abbreviations: CfDNA, circulating cell-free DNA; CA19–9, carbohydrate antigen 19–9; CA242, carbohydrate antigen 242; ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; BEAMing, beads, emulsions, amplification, and magnetics; OS, overall survival; PFS, progress-free survival; DSS, disease specific survival; HR, hazard ratio; CI, confidence interval; ARMS PCR, amplification-refractory mutation system polymerase chain reaction

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https://doi.org/10.1016/j.gene.2018.09.029
Received 27 July 2018; Accepted 14 September 2018
Available online 15 September 2018
0378-1119/

9), carbohydrate antigen 242 (CA242) are useful to monitor the prognosis of pancreatic cancer (Ni et al., 2007; Kawa et al., 1994; Hammad et al., 2010). However, some advanced pancreatic tumors may not cause abnormal elevations, and there exist false-positive results in hepatobiliary and gastrointestinal diseases. It has not reached consensus on the prognostic role of tumor markers. Recently, the circulating cell-free DNA (cfDNA) had got more attention as a promising prognostic biomarker for cancer, including colorectal cancer, lung cancer, breast cancer, etc. (El Messaoudi et al., 2016; Camps et al., 2011; Iqbal et al., 2015). The cfDNA are short nucleic fragments which can be isolated from the plasma or serum by non-invasive procedures. The cfDNA is released from apoptotic cells and remains consistent level in healthy people. However, due to the high cell turnover rate of tumors, the release of cfDNA would increase, and the genetic alterations of cfDNA are also more likely to be positive in cancer patients. When released from tumor cells, the cfDNA is also called circulating tumor DNA (ctDNA) (Yan and Song, 2016).

In terms of pancreatic cancer, cfDNA was also considered as a significant prognostic factor. Several studies have revealed that mutations, concentration, hypermethylation and ctDNA presence of cfDNA were associated with cancer progression and survival of pancreatic cancer patients (Chen et al., 2010; Cheng et al., 2017; Earl et al., 2015; Hadano et al., 2016; Kinugasa et al., 2015; Tjensvoll et al., 2016; Pietrasz et al., 2017; Semrad et al., 2015; Van Laethem et al., 2016; Castells et al., 1999; Singh et al., 2015). However, some studies indicated that they were not significantly related (Singh et al., 2015; Nakano et al., 2018), and the prognostic value of cfDNA for pancreatic cancer has not been systematically assessed. Therefore, we performed a meta-analysis to estimate the association between cfDNA and overall survival (OS) and progression-free survival (PFS) in patients with pancreatic cancer.

2. Materials and methods

2.1. Search strategy

Related articles were searched in PubMed and Embase databases until July 15, 2018. The detail search strategy were "(((((((cell-free DNA) OR cfDNA) OR cell free DNA) OR circulating DNA) OR ctDNA) OR kras)) AND ((pancreatic cancer) OR pancreatic carcinoma) AND ((((prognosis) OR prognostic) OR predict) OR predictive)".

2.2. Study inclusion and exclusion criteria

After scanning the titles and abstracts, we excluded the following articles at first: non-English language articles, case reports, reviews, comments and editorials. Studies would be included based on the following criteria: (1) studies included patients with pancreatic cancer, (2) studies had blood tests for cfDNA, and (3) studies analyzed the prognostic value of cfDNA for patients' survival results. We excluded some articles and confirmed the estimated selections according to our exclusion criteria: (1) cfDNA extracted from tumor tissue, (2) without survival result such as OS and PFS, (3) lacked key data for extracting HR, or (4) diagnostic articles.

2.3. Data extraction

Studies were accessed by two investigators (Yuan Cheng and Dan Zhang) respectively, and the disagreements were settled by consensus. Data extracted from the included articles contained first author's name, year of publication, country, number of patients, TNM stage, sample origin, time of sample collected, methods of DNA detecting, detecting markers, information about articles' quality. TNM stage is a notation system that describes the stage of solid tumor with alphanumeric codes. T classification describes the size of the original (primary) tumor and whether it has invaded nearby tissue. N classification describes nearby (regional) involved lymph nodes. M classification describes distant metastasis (spread of cancer from one part of the body to another). The survival data included overall survival (OS), progression-free survival (PFS) and disease specific survival (DSS). In the meantime, hazard ratio (HR), p value, the Kaplan-Meier survival curves and 95% confidence interval (95%CI) were also obtained from the related articles.

2.4. Quality assessment

We used the Newcastle-Ottawa scale (NOS) to score the quality of included studies (Stang, 2010). Specific score of each study was made sure after discussion. The scores of each study were consisted of 8 items with full mark of 9 scores. The studies with > 6 scores were considered as high-quality article in our meta-analysis (Parmar et al., 1998).

2.5. Statistical methods

The log Hazard Ratio (logHR) and standard error (SE) were used to present the results, which can be directly extracted from included articles. For articles without specific data of HR and 95%CI, we calculated the logHR and SE by extracting survival rates via Engauge Digitizer version 4.1 (Parmar et al., 1998). If the HRs were analyzed in both univariate and multivariate ways, respective pooled results would be presented. We also analyzed HRs in subgroup categorized by patient size, TNM stage, methods of DNA detecting, sample origin, time of sample collected and detecting marker. Our calculation work was conducted on the software projected by Matthew Sydes (Medical Research Council Clinical Trials Unit, London, UK), and Tierney et al. (2007).

According to Higgins and Thompson (2002), the heterogeneity was defined as $I^2 > 50\%$ or p value < 0.10. If the heterogeneity was not significant, a fixed effect model would be used; or else, we would choose a random effect model (Higgins and Thompson, 2002). The weight of each study was calculated by inverse variance method and adjusted by effect models in RevMan5.1, which determined how much each study contributed to the pooled HR. A HR > 1 without 95% CI overlapping 1 was statistically significant, which means the experimental group have worse outcome compared to the control group. All the above analyses were presented by RevMan5.1. And we use the Begg's funnel plot to estimate publication bias on STATA 11.0, the $p > 0.05$ indicated that significant publication bias was not existed (Priori et al., 2012). The "trim and fill" method were performed to further estimated the potential effect of publication bias in this meta-analysis (Willi et al., 2007).

3. Result

We extensively scanned a total number of 724 articles from PubMed and Embase databases. Among them, 678 articles were excluded after duplicates removed and records screening. After full-text reviewed, 28 articles were excluded due to cfDNA extracted from tumor tissue, without survival result such as OS and PFS, lacked key data for extracting HR or diagnostic articles. Therefore, only 18 articles (Chen et al., 2010; Cheng et al., 2017; Earl et al., 2015; Hadano et al., 2016; Kinugasa et al., 2015; Tjensvoll et al., 2016; Pietrasz et al., 2017; Semrad et al., 2015; Van Laethem et al., 2016; Castells et al., 1999; Singh et al., 2015; Henriksen et al., 2017; Kim et al., 2018; Nakano et al., 2018; Perets et al., 2018; Lin et al., 2018; Adamo et al., 2017; Ako et al., 2017) (1243 patients) were eligible for our meta-analysis. The procedure of selection was presented in Fig. 1. In addition, all the main characteristics of the eligible articles were summarized in Table 1. The mean patient number of studies was 69. The blood samples were all collected before treatment except two (Van Laethem et al., 2016; Nakano et al., 2018). Among them, 5 articles (Tjensvoll et al., 2016; Semrad et al., 2015; Van Laethem et al., 2016; Kim et al., 2018; Nakano et al., 2018) had survival outcome of PFS, one article had DSS data (Adamo et al., 2017). Molecular techniques such as droplet digital PCR