Review Article

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Expression of telomerase & its significance in the diagnosis of pancreatic cancer

Anutebeh Verdo Zisuh, Tian-Quan Han & Shen-Dao Zhan

Department of General Surgery, Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine, Sinan Road, PR of China

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Pancreatic cancer has one of the worst prognoses among all types of cancers. The survival rate is less than 5 per cent; this is due to difficulty in diagnosing at an early stage. Despite the improvements in diagnostic imaging techniques such as computed tomography, magnetic resonance imaging, *etc.*, the early diagnosis of pancreatic cancer is still difficult. Alternative methods of diagnosing pancreatic cancer at an early stage are presently been explored. The detection of telomerase activity has been proposed to be a useful tool in the diagnosis of pancreatic cancer. Telomerase is made up of three major parts namely, human telomerase reverse transcriptase, human telomerase and telomerase -associated protein. Several researchers have shown telomerase activity in tissues and fluids of patients with pancreatic and other types of cancers. About 95 per cent telomerase activity has been detected in pancreatic adenocarcinoma. Since telomerase activity is present in a vast majority of human cancers, it might have a role in the diagnosis and treatment of cancer.

Key words Diagnosis - pancreatic cancer - telomerase - telomerase activity - telomere

Pancreatic cancer is known to be the fifth most common cause of cancer death in the world with the lowest survival rates[1] less than 5 per cent due to the difficulty in diagnosing pancreatic cancer at an early state and partly due to the inaccessibility of the pancreas and its surrounding organs. Pancreatic cancer is highly metastatic. Clinical symptoms only surface when the cancer is already at its late stage and there is no specific symptom for pancreatic cancer.

Despite the fact that chemotherapy has improved the prognosis in many malignancies, its effect on pancreatic cancer is very limited. Pancreatic cancer has been found to be resistant to all anticancer drugs currently available[2] leaving surgical resection the only effective method to treat pancreatic cancer. But the quality of life of the patient is greatly hampered after the operation.

Although several improvements have been made in diagnostic imaging such as computed temography (CT), magnetic resonance imaging (MRI) magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), cancer markers such as CA199, CA242 *etc*. yet, the early diagnosis of pancreatic cancer remains very difficult. ERCP is presently being used to distinguish pancreatic cancer from other non-malignant

disorders. One of the shortcomings of the ERCP is that it is not capable of distinguishing pancreatic cancer from chronic pancreatitis, due to the similarity in the features. ERCP is also not useful in the evaluation of malignant potential of intraductal papillary mucinous neoplasm (IPMN). As a result, alternative methods for diagnosing pancreatic cancer are presently being employed such as DNA mutation, cytology K-ras, p53 gene overexpression and telomerase activity in pancreatic juice. The detection of telomerase activity in pancreatic cancer has been proposed as a useful tool in the diagnosis of pancreatic cancer[3,4].

Telomere and telomerase

Telomere is the protective DNA-protein complex found at the end of eukaryotic chromosome. Telomere DNA is made up of repeats of a simple, often G-rich sequence 5'TTAGGG3'[5]. Telomere shortens upon each cell division due to the DNA end replication problem and random breaks produced by free radicals. Telomere shortening may represent a "mitotic clock" associated with cellular senescence. Complete replication of telomeric DNA requires telomerase[6]. Telomere functions to prevent chromosome fusions. The capping function of telomere can also help protect chromosome ends against uncontrolled nucleolytic activity. Excessive telomerase activity is also controlled by the capping function of telomere.

The stability of telomere is ensured by the retention of sufficient DNA reserves and the activities of numerous protein including the shelterin complex such as telomeric repeat binding factor (TrF1, TRF2), telomere associated protein (TIN2), tripeptidyl peptidase (TPP1), protector of telomere (POTI) and repressor activator protein (RAP1). Presently, unlike RAPI, the roles played by TrF1, TRF2, TIN2, TPP1, and POTI in mammalian telomere stability has been well established[7]. The function of RAPI in telomere stability has not yet been established. The role of TPP1 in regulating TERT (telomerase reverse transcriptase) at the end of chromosome has been well established[8].

Telomerase is a ribonucleoprotein enzyme that catalyzes the synthesis of telomeric DNA. It therefore, helps in the formation and protection of telomere and also prevents cells from undergoing senescence[9,10]. Telomerase synthesizes the telomeric DNA strand running 5' to 3' towards the distal end of the chromosome thereby extending it. Telomerase is necessary for genomic stability. Fusion of a telomere with another telomere constitutes a catastrophic event for genomic stability. Telomerase acts to prevent this fusion.

Telomerase activity is commonly found in immortalized carcinoma cells and can also protect cells from apoptotic cell death[11]. The extent to which telomerase activity is regulated in cancer cells is not well known. Telomerase regulates telomere length, thus by changing the level of functional telomerase the length of telomere can be manipulated.

Telomerase is made up of three major parts namely, human telomerase reverse transcriptase (hTERT)[12], human telomerase RNA (hTR)[13] and telomerase-associated protein 1 (TP1) and telomerase-associated protein 2[14]. Among these only the expression of hTERT has been shown to correlate closely with telomerase activity. It, therefore, could serve as a parallel sign in the diagnosis and prognosis of pancreatic as well as other types of cancers. hTERT has been discovered to regulate telomerase activity, the expression of the catalytic subunit hTERT is crucial for telomerase activity[15]. hTR has been found to inhibit telomerase activity in tumour leading to telomere length reduction and the senescence of cells. hTR has been detected in both cancerous cells and non cancerous cells. Therefore, detection of hTR alone cannot play a major role in the diagnosis of pancreatic cancer. TP1 is also found in both tumour cells and non cancerous cells, and the presence of TP1 does not directly reflect the level of telomerase activity. Telomerase activity is found mainly in reproductive cells[16]. Telomerase activity is repressed in somatic cells except the haematopoietic progenitor cells, skin basal cells, intestinal stem cells and activated lymphocytes.

Telomerase is one of the attractive targets for anticancer research and therapy. Imetelstate (a telomerase inhibitor) is currently in the phase 1 and phase 2 trials[17]. Shay *et al*[18] observed telomere shortening after prolonged treatment with imetelstat.

Telomerase activity in pancreatic cancer

Telomerase activity has been reported to be present in pancreatic juice samples of patients with pancreatic cancer[19]. In a study about 95 per cent telomerase activity has been detected in pancreatic adenocarcinoma[20]. Kim *et al*[21], facilitated the research on telomerase activity in pancreatic juice of pancreatic cancer patients by the discovery of telomerase repeat amplification protocol (TRAP) and hybridization protection assay (HPA). TRAP and HPA combined together have been shown to

have a higher sensitivity. TRAP assay has not yet been applied clinically because of its complexity and its time consuming nature. C-circle assay (partially single stranded closed circular DNA molecules containing telomeric repeat tracts) detects higher C-circle (DNA circle) levels in blood from ALT (alternative lengthening of telomere) positive patients compared with ALT-negative, and may in future be applied clinically for cancer diagnosis[22].

The detection of telomerase subunit hTERT mRNA in pancreatic juice has been seen as a promising diagnostic tool not only for pancreatic cancer but also for other types of cancer[23]. The low level of telomerase activity has also been seen in non malignant pancreatic disorders such as benign adenoma, acute and chronic pancreatitis. The detection of telomerase activity in pancreatic juice has been found to be a promising diagnostic tool for differentiating between malignant and non malignant intraductal papillary mucinous neoplasm (IPMN)[24]. This will be a great break-throught because the present diagnostic tools we currently have are not able to distinguish between malignant IPMN and non malignant IPMN.

Telomerase activity has been detected in a patient 19 months before he was diagnosed as having pancreatic cancer[25]. Telomerase activity examined in both pancreatic cancer specimens and metastasis lesions, showed higher telomerase activity in metastasis lesions than in primary tumours. It is believed that telomerase activity is directly proportional to the age of the cancer. Telomerase activity may be an indication for a late event in carcinogenesis[26]. The level of telomerase activity in ductal cell carcinoma has been found to be significantly higher in comparison to those in other types of pancreatic cancer[27].

Nakashima *et al*[28] did a large scale analysis of 115 pre-operative pancreatic juice specimens to evaluate the feasibility of detection of hTERT expression by immunohistochemistry for pre-operative diagnosis of pancreatic malignancy, hTERT expression was detected in 84 per cent of pancreatic ductal adenocarcinomas (PDACs), whereas 62 per cent of PDACs were positive by cytology. When they combined the results of cytology and hTERT, the sensitivity and overall accuracy increased to 92.0 and 87.8 per cent, respectively. Hashimoto *et al*[29] detected telomerase activity in 83 per cent of patient with invasive ductal adenocarcinomas (IDCs) while hTERT was expressed in 88 per cent of IDCs. This show that detection of hTERT may be a better marker than telomerase activity.

hTERT expression and telomerase activity are predictors of poor outcome in pancreatic cancer[30]. No significant correlation has been found between the tumour side and the levels of both telomerase activity and hTERT.

Telomerase activity in bile

Telomerase activity has been detected in bile samples obtained from patients with pancreatic head cancer especially those with obstructive jaundice. The detection rate was 50 per cent lower than the rate in pancreatic juice. It is believed that bile may inhibit telomerase activity[31], therefore, to detect telomerase activity in bile duct cancers, it is better to detect telomerase activity in tissues.

When telomerase activity was detected in both pancreatic juice and resected pancreatic tissues, it was lower in pancreatic juice than that in resected tissues. This might be due to the fact that various digestive enzymes found in pancreatic juice, might have intervened in the telomerase assay in inhibiting Taq polymerase[32].

Telomerase activity in other types of cancer

Telomerase activity has been detected in different types of cancers[33]. It has been proposed that telomerase assays should be used in pre-operative investigation of various malignancies[34]. Hiyama *et al*[35] suggested that telomerase activity may be used to scrutinize equivocal biopsies. The detection of telomerase activity in different types of cancer may be helpful in predicting their biologic behaviour[36].

Problems encountered

In detecting telomerase activity in pancreatic juice and pancreatic tissues, researchers have encountered problems in obtaining pancreatic juice during operation, or during ERCP. This is an important disadvantage because sample reliability and higher sensitivity and specificity of the assay are key preconditions for its clinical use in the diagnosis of pancreatic cancer.

When normal and cancer tissues are taken for the detection of telomerase activity, sometimes telomerase activities are detected in normal tissues. This might be due to cancer penetration into the normal tissues. Sometimes false positive results are obtained due to contaminated lymphocytes which can show telomerase activity without malignant transformation. Kim *et al*[21]

detected hTERT in CD25-positive but not in CD25 negative, peripheral lymphocyte in a normal healthy volunteer. Therefore, in order to avoid the false positive result caused by the contaminated lymphocytes, they suggested the removal of samples exhibiting CD25 expression. Also, TRAP assay is not suitable for clinical use because of its complexity and time consuming factor and also the difficulty in obtaining qualitative and quantitative samples for telomerase analysis. Another difficulty is that it involves the use of radioisotopes.

Conclusion

Since telomerase activity is present in a vast majority of human cancers, it may have a clinical application in diagnosing and treating cancers. The majority of pancreatic cancers shows telomerase activity, consequently, the detection of telomerase activity using a modified TRAP assay may support a diagnosis of pancreatic cancer. Since C-circle assay is capable of detecting higher levels of C-circles in blood from ALT-positive patients compared with ALT-negatives, it may in future be applied clinically for cancer diagnosis. Telomerase can be exploited as a target to diagnose and treat cancers because several studies showed telomerase inhibition resulting in telomere instability and cell death[37,38].

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Reprint requests: Dr Tian-Quan Han, Department of General Surgery, Ruijin Hospital affiliated to Shanghai Jiaotong, University School of Medicine, Sinan Road 27, 200025, PR of China
e-mail: anudo2@yahoo.com