LOSS OF MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 4 (SMAD4) EXPRESSION AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN PANCREATIC CARCINOMA

Autores

C. Vareda - Instituto Politécnico de Coimbra; Escola Superior de Tecnologia da Saúde de Coimbra, *BSc*
P. F. Teixeira - Centro hospitalar e Universitário de Coimbra, *MSc*
D. R. Martins - Instituto Politécnico de Coimbra; Escola Superior de Tecnologia da Saúde de Coimbra, *PhD*
A. M. Valado - Instituto Politécnico de Coimbra; Escola Superior de Tecnologia da Saúde de Coimbra, *PhD*
F. J. Mendes - Instituto Politécnico de Coimbra; Escola Superior de Tecnologia da Saúde de Coimbra, *PhD*

Centro de execução do trabalho
Instituto Politécnico de Coimbra; Escola Superior de Tecnologia da Saúde de Coimbra

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Contacto do autor responsável
catarinavareda@gmail.com

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Abstract

Pancreatic cancer (PC) has an important role in the clinical and research area representing one of the lowest five-year rates as well as a global mortality rate of 4.8% due to its late and poor diagnosis. Therapeutic strategies have also an unsatisfactory response. Even after surgery, the recurrence or appearance of metastasis are frequent, leading to a poor overall survival.

The PC has been related with several mutations, including K-RAS; P16; TP53; HER2. Besides, it is also associated with the deleted in pancreatic cancer locus 4 (DPC4), also known as the suppressor mothers against decapentaplegic homolog 4 (SMAD4) which is present in nearly 50% of the diagnosed patients with PC.

Preceding studies proved that SMAD4 loss expression plays an important role in tumorigenesis and in the promotion of pancreatic carcinoma´s growth. Therefore, it is highly relevant in late stages suggesting that SMAD4 may be a molecular biomarker in prognostic results.

The main goal of this review is to highlight the foregoing findings focused on SMAD4 deletion and its influence in clinicopathological parameters in pancreatic carcinoma by referring some of the investigations and clinical trials made in this field. Furthermore, it is also required to contemplate some of the therapeutical strategies and the influence of SMAD4 in future therapies.

Keywords
Pancreatic cancer; SMAD4; TGF-β

Introduction

The deleted pancreatic cancer locus 4 (DPC4), also known as the suppressor mothers against decapentaplegic homolog 4 (SMAD4), plays a major function in the carcinogenesis of the pancreatic ductal adenocarcinoma (PDAC) [1]. The PDAC represents one of the most common types of pancreatic cancer (PC) as approximately 90% of all pancreatic malignancies [1,2].

SMAD4, located in chromosome 18q21.1, is a suppressor gene that significantly impacts the transforming growth factor beta (TGF-β) pathway signalling regulation [3]. This downstream effect has an essential role in the proliferation and survival of tumour cells [3]. The loss of expression of the SMAD4 has a higher frequency in pancreatic carcinoma and a lower percentage in other types of carcinomas as breast, ovary, stomach, oesophagus, neck, colon, and biliary tract [3].

This gene can be inactivated by homozygous deflection due to the deletion of both alleles or just the mutation in one of the alleles, consequently leading to the loss of the other (heterozygosity) (3). Wilentz et al. used 46 tissue samples from PC to demonstrate that immunohistochemistry labelling for SMAD4 is a specific and sensitive method to detect SMAD4 inactivation by either homozygous or heterozygous deletions [4].

SMAD4 gene

In 1996, Hahn and his team did the first description of SMAD4 and its repercussions in PC [5]. The SMAD4 has 10 introns and 12 exons [5,6]. Preliminary studies identified 11 exons, and later it was discovered another exon named exon 0 [5,6]. This gene encodes a 552 aminoacids protein with 60 KD as its molecular weight [5,6].

Structurally, this protein involves three main components: N-terminal MH1 domain and C-terminal MH2 domain linked by an intermedial region [1]. This region´s function is to recognize the SMAD-binding element that can bind DNA and interact with other SMAD protein through MH1 domain [1]. To activate transcriptional activity, it is necessary the presence of SMAD activation domain located in C-terminal of the linker region [1].

This region owns phosphorylation spots for the mitogen-activated protein kinase (MAPK) and the extracellular signal-regulated kinases (Erk) [4]. Additionally, it possesses spots of consent for kinases regulated by calcium and one proline-tyrosine motif, that recognizes WW domains for sumoylation and ubiquitination of SMADS (4).

The homozygous deletion in PC is present in approximately 30% of the cases [4]. The repercussions of SMAD loss starts in late stages of the neoplasia and it is only histologically detectable in advanced infiltrative stages [7].

In later investigations, Wilentz and their team observed a significant difference between low-grade and high-grade neoplasms in PC by comparing histological features labelled with monoclonal antibody for SMAD4 [8]. They concluded that the loss of SMAD4 gene appears in late stages of tumorigenesis and helps the progress and tumour invasion at a histological identifiable level [8].

The SMAD4 protein

SMADS family

SMAD4 is one of the eight members of the SMAD family, which is divided into three sub-groups [11]. The R-SMADS, sub-group also known as the receptor-regulator SMADS which includes SMAD 1, 2, 3, 5 and 8 [11]. The co-SMAD4 or just SMAD4 represents the third group [11]. The last sub-group is the I-SMADS that includes SMAD 6 and 7 which have inhibitory functions [11].

The mechanisms of SMADS centres on the phosphorylation and activation through transmembrane serine-threonine receptor kinases responding to TGF-β [11]. SMAD4 can either form homomeric or heteromeric complexes that interact with other activated SMADS, leading to an accumulation in the nucleus, interfering with the transcription of specific genes [11].

SMAD4 protein location

The SMAD4 protein can move between the nucleus and the cytoplasm [9,10]. It can break through the nuclear membrane in a spontaneous process that is not dependent on TGF-β signalling [9,10]. The immunohistochemistry mainly presents a cytoplasmatic staining [9,10]. However, if the gene is intact, the nuclear staining can be used too [9,10].

In laboratory diagnosis, immunohistochemistry is a useful method to distinguish between in situ and invasive PDCA on biopsies samples of benign to reactive pancreas [9,10]. In benign lesions, the SMAD4 immunoexpression is still present [9,10].

SMAD4 in pancreatic cancer

SMAD4 and the TGF-β pathway signalling

TGF-β is a major signalling pathway involved in PDCA [1]. A previous study reported that increased levels of TGF-β in the serum is an indicator of poor prognosis related to a weak survival in unresectable tumour cases [1].

In a normal physiological response, SMAD4 interacts with TGF-β to prevent tumorigenesis [7]. This partnership results in the blockade of mitogenic growth signals [7]. This blockade leads to the inhibition of cell proliferation as well as the activation of programmed cell death via apoptosis of the pancreatic cells [7]. In other words, the TGF-β/SMAD4 signalling pathway restores the balance and homeostasis ensuring the tumour suppressive environment [7].

In a tumoral state, the deleted SMAD4 associated with a TGF-β mutated pathway creates a deregulation of the transcriptional phase boosting the proliferation and the cell expansion of the malignant cells [5].

Levy et al. inhibited SMAD4 functions in cell lines Colo-357 of pancreatic tumour using a tetracycline-inducible small interfering RNA [12]. They proved that the loss of SMAD4 can stimulate tumorigenesis by eliminating the normal TGF-β/SMAD4 pathway with a tumoral suppressor role [12].

This pathway also controls the communication between the tumour and the stroma [13]. PDAC is subcategorized into two main components: the complete epithelial-mesenchymal transition (EMT) and the partial EMT [13]. It is suggested that the last one may be a product of increased metastasis rate [13]. When TGF-β/SMAD4 is compromised, the modulation of fibrotic response and intracellular mechanisms suggests that this mutation promotes the proliferation and changes the metabolic programme in tumour microenvironment [13].

Therefore, SMAD4 have a dual mechanism in the tumour, indicating the possibility to inhibit TGF-β rather than activating [14]. Pre-clinical trials have revealed a possible TGF-β inhibitor (Galuni-sertib) showing efficacy when combined with chemotherapeutics [14].

Besides, it is also known that SMAD4 down-regulates the expression of proto-oncogene c-Myc and up-regulates p15 and p21 (CDK inhibitors) [6]. SMAD4 is also involved in various events like apoptosis, angiogenesis, differentiation, and cell cycle regulation [6].

SMAD4 and the tumour microenvironment

The tumour microenvironment (TME) is portrayed as an important piece in the neoplasia progression and cell growth, providing a suppressive ambience [15]. It is formed by a dense stromal compartment with cancer-associated fibroblast, blood vessels and infiltrating immune cells [16]. Patients diagnosed with PDAC and complemented with a decidedly immunosuppressive profile were more likely to have a poor prognostic [15].

Wang et al, proved the existence of a significant correlation between infiltrating immune cells in the tumour microenvironment and the suppressive genes like TP53; p16 and SMAD4 [15]. They have a specific influence on the overall survival and in the relapse-free survival (RFS) [15]. They used different assays like immunohistochemistry and quantification of gene expression which revealed that SMAD4 performs an important role in recruiting and regulating infiltrating immune cells in the TME [15].

SMAD4 in the PC development and progression

Three main neoplasia lesions have been described by being potentially involved in the malignancy progression defined as: pancreatic intraepithelial neoplasm (PanIN), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) [17,18].

PanIN is the most frequent and the most well-known neoplasia lesion [17,18]. It is divided into three main categories: PanIN-1 which has no cellular atypia, PanIN-2 lesion characterized by cellular atypia and the papillary architecture [17,18]. The PanIN-3 resembles to carcinoma in situ [17,18]. It has been reported that the loss of SMAD4 appears to be more frequent in PanIN-3 and in PDAC when compared to other types of pancreatic cancer [17,18].

It is important to consider that SMAD4 mutation alone is insufficient for the development of PanIN-1 [17,18]. Subsequently, the malignancy evolution is based on several suppressing genes and pro-oncogenes activation, providing a perfect genomic ambience for tumorigenesis [17,18].

Clinicopathological parameters and therapy strategies

The current treatment of pancreatic cancer is based on surgery, chemotherapy, radiation and palliative care, depending on the stage [19].

The final diagnosis is based on different parameters like tumour stage including the T stage, the presence of lymph nodes metastasis as well as distant metastasis and other imaging exams [20]. A study revealed that the complementary analyses are beneficial to establish a more accurate diagnosis [20]. Several therapeutical strategies, clinicopathological analysis and laboratory parameters were evaluated as potential prognostic factors compared with short and long-term survival [20]. The analysis included increased levels of total bilirubin, higher levels of CA19-9, advanced T stage, the existence of lymph node metastasis and lack of surgical and chemotherapy procedures [20]. They concluded that evaluation of the prognostic factors was linked to worse outcomes validating the importance of the independent prognostic parameters [20].

Currently, the therapy strategies are applied based on the different tumoral stages [21]. In resectable tumours, the gold standard is the surgery with adjuvant chemotherapy (gemcitabine and capecitabine) [21]. The neoadjuvant strategies are more applicable to chemotherapy than radiation therapy in cases of a borderline, locally advanced or unresectable tumours. Nevertheless, more comprehensive studies are needed in this field [21].

For metastatic tumour, the FOLFIRINOX and nab-paclitaxel–gemcitabine are used, presenting a better rate of survival compared with monotherapy [21].

SMAD4 and prognostic factors

Throughout this review, it is understood that the loss of SMAD4 cooperates with neoplasia progression and promotion of tumour growth. Therefore, many studies have been made to correlate the mutation with clinical and prognostic parameters as well as understanding its association with therapy responses.

A previous study compared the effectiveness of treatment strategies for recurrent PDAC with the SMAD4 genetic status by analysing recurrence patterns and their responses to several therapeutical strategies [4]. Outcomes indicated that recurrence patterns after pancreatectomy rely on SMAD4 genetic status [4].

Later, Shin et al. studied 641 patients with recurrent PDCA combined with chemotherapy as well as local control [22]. They concluded that the inactivation of the SMAD4 gene was a major parameter to predict metastatic reappearance [9]. Patients with an initial loss of SMAD4 had a better response to intensive local control of relapses, which was a major factor to choose a higher effectiveness initial treatment for recurrent PDAC [22].

In opposition, specialists in the field suggested that negative SMAD4 pancreatic carcinomas have the worst response to treatment for distant metastasis [23]. Therefore, chemotherapy is more suitable for locally advanced PDAC [23].

Since TGF-β/SMAD4 have a crucial role in carcinoma progression, it is a great potential target for therapy [7]. Clinical trials in mouse models showed the neutralized TGF-β type III receptor (TBRII) could decrease metastasis and tumour proliferation and at the same time, it would increase apoptosis in PDAC primary tumour [7].

Prospective therapies

Despite some controversial studies, SMAD4 is still a budding target for therapy [24]. Thus, several studies have been made to accomplish some novel treatments [24].

In addition to TGF-β mutated pathway, there are other altered pathways such as WNT/GSK3 and ERK pathways [24]. These alterations can boost the glycogen synthase kinase 3 (GSK3) phosphorylation resulting in protein degradation and the subsequent loss of SMAD4 function [24]. Later investigations revealed that is possible to use a GSK3 inhibitor to restore TGF-β pathway and stabilize SMAD4 [24].

Another investigation suggested that the detection of this mutation may be helpful to stratify patients for a better choice in the therapeutical protocols [24]. The researchers used screening methodologies based on synthetic lethality to detected two components named as UA62001 and UA62784 [24]. So that, they could target selectively the negative SMAD4 cells [24]. The cells treated with UA62001 showed an interruption of the cell cycle during phase S and G2 (mitotic phases) [24]. Additionally, the UA62784 component activated the CDK1 and generated mitotic cell arrest and apoptosis [24].

Conclusion

Despite all the improvements made in this topic, there are still some uncertainties on the real influence of SMAD4 loss of expression in clinicopathological findings for pancreatic cancer. This reinforces the need for new advances in this area. There are yet many challenges ahead, such as the poor overall survival associated with a late prognosis regardless of the carcinoma stage.

Nevertheless, SMAD4 remains a potential biding target for therapy giving hope to future investigations and optimistically bringing an upturn of the overall survival for patients with pancreatic carcinoma.

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