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EDITED BY
Liang Qiao,
Westmead Institute for Medical Research, Australia

REVIEWED BY
Francisco X. Real,
Spanish National Cancer Research Center (CNIO), Spain
Rocco Mazzolini,
Centre de Regulació Genòmica, Spain

*CORRESPONDENCE
Carles Barceló
carles.barcelo@ssib.es
Catalina M. Perelló-Reus
catalinamaria.perello@ssib.es

†These authors have contributed equally to this work and share first authorship

‡Senior last author

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Challenges in precision medicine in pancreatic cancer: A focus in cancer stem cells and microbiota

Catalina M. Perelló-Reus[1*†], Teresa Rubio-Tomás[2†], Eugenia Cisneros-Barroso[3], Lesly Ibargüen-González[1], Juan José Segura-Sampedro[4,5], Rafael Morales-Soriano[4,5] and Carles Barceló[1*‡]

[1]Translational Pancreatic Cancer Oncogenesis Group, Health Research Institute of the Balearic Islands (IdISBa), Hospital Universitari Son Espases (HUSE), Palma de Mallorca, Spain, [2]School of Medicine, University of Crete, Herakleion, Greece, [3]Internal Medicine Department, Son Llàtzer University Hospital, Palma de Mallorca, Spain, [4]Advanced Oncological Surgery, Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain, [5]General and Digestive Surgery Unit, Hospital Universitari Son Espases, School of Medicine, Balearic Islands Health Research Institute, University of Balearic Islands, Palma de Mallorca, Spain

Pancreatic cancer adenocarcinoma (PDAC) is a lethal disease, with the lowest 5-years survival rate of all cancers due to late diagnosis. Despite the advance and success of precision oncology in gastrointestinal cancers, the frequency of molecular-informed therapy decisions in PDAC is currently neglectable. The reasons for this dismal situation are mainly the absence of effective early diagnostic biomarkers and therapy resistance. PDAC cancer stem cells (PDAC-SC), which are regarded as essential for tumor initiation, relapse and drug resistance, are highly dependent on their niche *i.e.* microanatomical structures of the tumor microenvironment. There is an altered microbiome in PDAC patients embedded within the highly desmoplastic tumor microenvironment, which is known to determine therapeutic responses and affecting survival in PDAC patients. We consider that understanding the communication network that exists between the microbiome and the PDAC-SC niche by co-culture of patient-derived organoids (PDOs) with TME microbiota would recapitulate the complexity of PDAC paving the way towards a precision oncology treatment-response prediction.

KEYWORDS

precision medicine, PDAC, cancer stem cell, niche factors, microbiota, early diagnosis, therapy resistance, organoid co-culture

Introduction

Precision medicine (PM) involves the customization of healthcare for a specific individual on the basis of biomarker measurements obtained at the individual and population levels (1). Remarkably, in the last years the management of cancers of the gastrointestinal system is moving towards a precision medicine paradigm (2) in which biomarkers for precision medicine are a topic of intense research (3, 4).

In the last decade it has been established the central role of cancer stem cells (CSC)- i.e. the subpopulation of cancer cells capable of self-renewing and producing progeny- in the progression, treatment resistance and metastasis of gastrointestinal cancer (5–7). CSCs depend on their niches, which are anatomically distinct regions within the tumor microenvironment (TME). These niches maintain the principal properties of CSCs, preserve their phenotypic plasticity, protect them from the immune system and facilitate their metastatic potential (8–10). Interestingly, biomarkers related to CSCs and its niche/TME have been found to be among the most accurate in prediction of disease progression and, specially, disease recurrence (11–13) and also to develop tailored therapies that optimize patient's opportunities to cure (14)

A variety of tumors contain bacteria what suggests that the microbiome could play a role in the TME (15). In fact, the microbiome is proposed to have an active involvement in the pathogenesis and treatment responses. This is in line with the view that tumors should be treated as biosystems instead of only a set of transformed epithelial cells (16). Specifically, microbiota-related biomarkers have recently been posed both as predictors of disease progression and treatment response (17), and as relevant targets of anti-cancer therapies in many malignancies (18). Thus, studying the interplay between cancer stem cells and intratumoral microbiota seems to be a promising strategy in the development of new biomarkers for a cancer precision medicine.

Challenges in PDAC personalized treatment

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease, with the lowest 5-years survival rate of all cancers (18) Although PDAC presents low frequency (incidence of 8–12 cases per 100 000 people per year, and a 1·3% lifetime risk for the disease) will be the second cancer-related death reason in 2040 in the USA (18). Despite the advance and success of precision oncology in gastrointestinal cancers, the frequency of molecular-informed therapy decisions in PDAC is currently neglectable (19)Therefore, understanding the pathogenesis of this lethal disease is urgently needed to stratify patients and to develop personalized novel therapeutic approaches for it.

Current therapies rely on conventional polychemotherapies with poor outcomes and molecular-informed targeted therapy opportunities only exist in a tiny minority of patients (19). This clearly demonstrates that the tremendous potential of genetically guided precision oncology used in other GI malignancies (2, 20), in PDAC meets important limitations. For that reason, there is the need to expand the knowledge about PDAC biology in order to decipher other targetable mechanism such as tumor microenvironment and cellular plasticity (5, 21)

Cellular plasticity is the ability of tumor cells to adapt to changing conditions by acquiring different molecular and phenotypic identities and, thereby, plasticity programs are key regulators of acquired treatment resistance (22).For this purpose, one of the most critical questions in both cancer research and clinic is how PDAC is maintained and expanded after it has emerged. Cancer stem cells (CSCs), and particularly PDAC stem cells (PDAC-SC) have been considered as a subpopulation of cancer cells capable of self-renewing and producing progeny cells that are critical for cancer growth (23, 24). This mechanism may underlie the maintenance of cancer and its resistance to conventional therapies.

According to the current model, CSCs are not a fixed cell population but a plastic one, i.e. the aforementioned characteristics can be acquired and lost dependent on environmental stimuli (25)Therefore, CSC are highly dependent on their niche, i.e. microanatomical structures of TME in which CSCs are maintained and protected from therapy (26, 27)

In this regard, in an unbiased approach, clonogenic capacity of PDAC-SC was shown to be fully defined by the microenvironment and not by tumor-cell-intrinsic-features (28) confirming a dichotomous role of stroma either promoting or inhibiting PDAC-SC tumorigenic capacity (29, 30)

We believe that the PDAC CSC (PDAC-SC) biology is strongly affected by the interplay between the genetic alteration and the tumor microenvironment, particularly the microbiome. Unraveling the link between microbiota and cancer stem cells technologies will provide insights into the pathology of cancers of the gastrointestinal system, as well as promote the translation of these findings to the clinics towards personalized medicine.

Late diagnostic

Given the dismal prognosis of PDAC patients, early and differential diagnosis of severe pancreatic cancers is essential and challenging for patients with PDAC and constitutes an unmet clinical problem (18). Symptoms are unspecific and often emerge only during late disease stages, at which point, tumors can be either locally non-resectable or present as metastatic disease. At present, PDAC is diagnosed using imaging tests and currently, despite other promising circulating biomarkers have been described (31) the sole FDA-approved biomarker for PDAC is serum CA19-9, mostly used for disease monitoring

rather than screening, due to inherent limits of sensitivity and specificity: CA19-9 levels can be elevated in several conditions unrelated to pancreatic cancer, while subjects lacking the Lewis-A antigen do not produce CA19-9 at all (32). Thus, the outcome of PDAC patients could improve with sensitive and affordable tests that would permit early detection of the disease.

A plethora of studies have shown that microbiota most likely affects the malignant phenotype and prognosis of PDAC (33, 34) Therefore, microbiome signatures enable robust metagenomic classifiers for PDAC detection at high disease specificity and with potential towards cost-effective PDAC screening and monitoring. Interestingly, in a recent study (35), showed that faecal metagenomic classifiers had much better performance than saliva-based classifiers and could identify patients with PDAC with an AUC score of up to 0.84 based on a set of 27 microbial species, with consistent accuracy across early and late disease stages, increasing when combined with serum levels of CA19-9, indicating the potential for non-invasive, robust, and specific faecal microbiota-based early diagnosis for PDAC (35)

Many studies suggest that quiescent plastic CSCs are already present but resting/latent during early stages of disease development (26, 36). Importantly, early quiescent PDAC-SCs initiate KRAS mutant pancreatic lesions leading to PDAC in the context of pancreatitis (37, 38) a condition known to be heavily influenced by microbiome (33). Interestingly, circulating PDAC circulating tumor cells with stem-like characteristics could be used as an early PDAC biomarker (39).

Therapy resistance

The accumulation of driver mutations is accompanied by histological changes that represent the different stages of PDAC development. Morphological evolution begins with the formation of precursor lesions, termed pancreatic intraepithelial neoplasia (PanIN), with increasing histological grades followed by progression to invasive adenocarcinoma (Figure 1A).

A histopathological hallmark of PDAC is a desmoplastic reaction to the tumor that is present in both primary and metastatic tumors (40). Pancreatic stellate cells, a myofibroblast-like type of cell in the pancreas are activated by cancer cells to produce high fibrosis surrounding the tumor (41).The resultant desmoplasia is known to be responsible for creating a mighty mechanical barrier around the tumor cells, preventing appropriate vascularization, and thus limiting exposure to chemotherapy and largely preventing immune cell infiltration (42)

Early research largely stemmed from the idea that the surrounding desmoplasia is tum or promoting but this view of its role is most probably an imperfect one (29). Therapeutic approaches to target stromal desmoplasia have classically focused on depleting the stromal constituents but results have been generally disappointing, owing to the multi-faceted nature of tumor stroma (43). Furthermore, TME composition is a cell-extrinsic factor that influences the transcriptional landscape. Depending on the mRNA expression two major tumor subtypes have been described: basal or classical (44, 45). Interestingly, basal subtype presents stem-like properties (46), which interestingly correlates with dismal prognosis (47) and poor gemcitabine response (48).

In this regard, the intestinal microbiome has recently gained increasing interest in the field of PDAC TME with studies suggesting a tumorigenic relevance of bacterial dysbiosis within the TME. Since the early evidence of bacteria presence in PDAC TME (16, 34) and despite substantial inter-individual variability of the gut flora, some studies concur in their findings, pointing at different bacterial species potentially involved in PDAC tumorigenesis thought their interaction with the desmoplastic stroma (49). The formation of a new desmoplastic niche that offers lower colonization resistance and provides nutrition in the form of increased glycan levels might favor the migration of specific bacteria (25, 50, 51). In turn, new resident bacteria might remodulate the TME to promote tumor development and progression by favoring a PDAC-SC niche refractive to chemotherapy by inducing EMT-dependent stemness state or metabolizing chemotherapeutic agents (25, 34, 51), processes that could even cooperate to enhance therapy resistance (52).

The most prominent, although not exclusive, microbes identified in pancreatic tissue samples and associated with PDAC TME are Gram-negative bacteria, more specifically from the phylum *Proteobacteria* (25, 50, 53). Among *Proteobacteria, Gammaproteobacteria* was associated with poor patient prognosis (34, 53). These bacteria express the enzyme cytidine deaminase which enables the metabolization of the chemotherapeutic drug gemcitabine (2′,2′-difluorodeoxycytidine), which is commonly used for treatment of PDAC patients in the adjuvant and palliative setting, into its inactive form (2′,2′-difluorodeoxyuridine) (40). This might synergize with a quiescent PDAC-SC subpopulation, able to evade chemotherapeutic anti-tumor therapies (54) which is a hallmark of plastic PDAC-SC and responsible for disease relapse years after successful surgical intervention or tumor free survival (55).

Importantly, a distinct tumor microbiome was shown to clearly discriminate long-term survivor (median survival: 9.66 years) from short-term survivor (median survival: 1.66 years) PDAC patients with a strong correlation between dismal prognosis and low diversity (56). Long-term-survivors (LTS) contain higher alpha-diversity, presenting also Gram positive classes such as *Clostridia* and *Actinomycetia*, and a LTS-specific intra-tumoral microbiome signature was described (56). Of note, elevated levels of single microbial species correlated with poor prognosis, making diversity analysis, even in the stools an attractive, cheap, and non-invasive method to predict prognosis (35, 57).

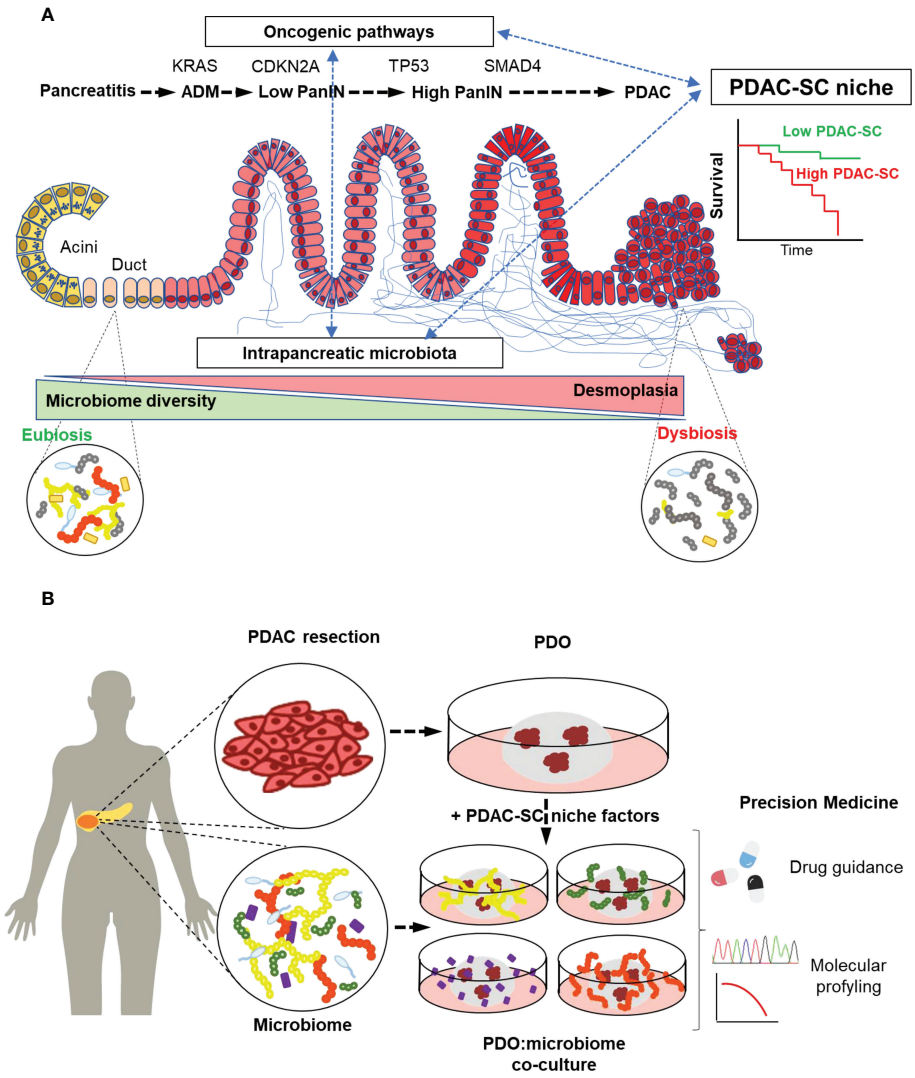

FIGURE 1
Intratumoral Microbiota may define PDAC stem cell niche, thereby constituting a diagnosis and prognosis biomarker. **(A)** In the development of PDAC, driver mutations accumulation is accompanied by an increasing desmoplastic reaction (blue lines) as a hallmark histopathological feature in PDAC stroma. Microbiota embedded in the desmoplastic stroma changes towards a dysbiotic low-diversity composition that might impact the PDAC stem cell niche by favoring tumor progression and resistance to chemotherapy. **(B)** Patient-derived organoids (PDOs) are generated mainly from PDAC resection containing PDAC cancer stem cells (PDAC-SC). PDOs can be co-cultured with the patient microbiota to recapitulate the PDAC-SC niche. This co-culture technique paves the way to the study of microbiome-focused precision medicine bench-to-beside approaches to overcome the lack of early diagnosis and therapy resistance in PDAC.

Personalized medicine in PDAC: A holistic ex vivo co-culturing modeling to predict treatment response

To follow a personalized medicine approach, there is an urgent need to find a model that recapitulates the tumor characteristics and that could be generated in a useful time frame taking into account the time constraints of PDAC management. Current models show limitations. PDAC derived 2D cell lines fail in reproducing the polarity, microenvironment, cell metabolism and gene expression which can affect drug response prediction (58). Patient-derived xenografts (PDX) better reproduce the tumor and predict drug response but the use of PDX remains challenging due to time concerns and the lack of a human microenvironment.

Alternatively, organoids are a 3D model derived from CSC that can be generated in 2-4 weeks and maintain the histological and genetic features of the tumor of origin. In 2013, Huch et al.

described for the first time the generation of pancreatic organoids (59). Several years later, the same model was used to generate PDAC organoids from mouse and human tissue defining the medium composition (60). This offers the possibility to design a personalized medicine approach by using primary PDAC patient material (tumor resection or fine needle biopsy) to generate patient-derived-organoids (PDOs) that can work as patient avatars to predict the therapy response. PDOs can be also generated from human induced pluripotent stem cells (61).

In that sense, different PDAC PDOs biobanks have been generated (30, 60, 62, 63). PDAC organoids recapitulate the mutation profile of the original tumor (62, 63) and have been demonstrated to be a valuable tool to test drug sensitivity (48, 62–64) that even allows the study of drug-induced vulnerabilities in tumor relapse that can be therapeutically exploited in a bench-to-bedside approach (19).

Nevertheless, PDOs present some limitations such as that the drug response can be modified by the transcriptional changes due to the bottleneck of medium composition. In fact, PDOs transcriptional landscape depends on culture conditions favoring certain subtypes (63). Furthermore, dependence on growth factors and medium composition can exert a selective pressure to select the organoids containing the driver mutations of PDAC (30).

The lack of physiological niche factors could be bypassed by the use of tumor-on-a-chip devices that reproduces the TME. This includes the incorporation of stromal cells (65, 66) what highlights the importance of the co-culture with other cell types in order to mimic the complexity of the tumor. This is particularly relevant in PDAC, a tumor characterized by its low cellularity. Organotypic co-culture models have been established in PDAC PDOs. In that sense, PDOs have been co-cultured with cancer associated fibroblasts (CAFs) (67–70) and infiltrating lymphocytes isolated from blood (67) Interestingly, in co-culture experiments, Lodestijn et al. demonstrated that the factors secreted by TME maintain populations of tumor cmells with clonogenic potential (28).This shows the importance of co-culture PDAC-SC with the stromal factors responsible for maintaining CSCs and/or promoting the dedifferentiation of non-CSC tumor cells.

Since tumor microbiome is clearly affecting PDAC oncogenesis (34, 53, 71–73) this could be considered another key TME element with an impact on PDAC drug screening. The addition of the purified tumor microbiome to the PDO models would add a layer of complexity to the *in vitro* modeling of this dismal prognosis disease, better reproducing the tumor characteristics anticipating a good predictive drug response tool. In particular, it could be interesting to study the effect of microbiome on PDAC-SC population. As far as we know, the co-culture of organoids and microbiome has not been done yet in PDAC but there are established protocols in intestinal models (74, 75). The microinjection of the bacteria into the lumen mimics the microbiome habitat (75). However, the manipulation of specific factors (e. g. oxygen and nutrient levels) to allow the co-culture of a diversity of bacteria with organoids remain to be established.

Co-culture experiments could be used to envisage the effect of tumor microbiota on CSCs. Furthermore, developing a model able to recapitulate the complexity of PDAC-SC niche (Figure 1B) paves the way towards a more accurate and physiological treatment-response prediction capacity of cultured PDOs.

Molecular studies to uncover microbiome- stem cell niche crosstalk

As stated before, *in vitro* models aiming to recapitulate the complexity of PDAC-SC niche need to include the microbiome axis to fully define the complex crosstalk between stem cells and microbiota.

The controlled escalation of biological complexity on the host side as well as in the composition of microbiome-derived secreted factors or live bacterial communities enable the proof-of-concept of a complex interaction mechanism in a controlled and standardized environment. These models open the door to a new generation of molecular studies difficult to study *in vivo*.

Microbial communities in the gut are known to produce small molecules and metabolites that significantly contribute to host functions and homeostasis (76). This interplay has been extensively studied in the intestine using microbiome-organoid co-culture models. In this regard, Sodhi et al. (28) found that bacterial Lipopolysaccharide (LPS) activates Toll-like receptor 4 (TLR-4) and enhances cell differentiation of goblet cell lineages in colonic organoids but inhibits Lgr5+ colon stem cells (77, 78) Similarly, a recent study found that dietary raffinose is utilized by *Lactobacillus reuteri* to convert it to fructose which in turn engages glycolysis to fuel stem cell proliferation under stress conditions (79).

As stated before, some bacterial families are conducive to oncogenesis and progression, while others prevent tumor development and might aid innate and therapeutically induced anti-tumor immunity. However, studying microbiome effects on tumor-related immunity in *ex vivo* systems is challenging, normally forcing the use of *in vivo* models which makes it difficult to dissect direct effects of microbiota on immune cells. Again, the use of microbiome-organoid co-culture approaches could circumvent the difficulties. In this regard, a recent study developed a novel immune-enhanced tumor organoid system to study factors affecting Immune Checkpoint Blockade (ICB) response. Selective testing of bacterial-derived metabolites from species found in the immunomodulatory host-microbiome significantly increased ICB-induced apoptosis of tumor cells and altered immune cell receptor expression (80).

Organoid have been used extensively to model senescence and aging-related conditions (81). In this regard, a recent study (82) found that gut microbiota metabolite trimethylamine N-oxide induces aging-associated senescent phenotype in midbrain organoids. Also, with these models, stem cell DNA damage associated to microbiota could be studied. Microbial co-culture with gastric organoids uncovered the mechanism by which *Helicobacter pylori* favours the accumulation of DNA damage promoting gastric cancer. In this regard (83), reported that DNA damage by *H. pylori* occurs in an ALPK1/TIFA/NF-KB-dependent manner in S-phase cells and importantly, the *H. pylori* LPS precursor (β-ADP-heptose) was sufficient to induce this damage.

Similar approaches could indeed be used to isolate individual microbiome-induced factors that alter PDAC-SC niche with the intrinsic limitation of the complexity of PDAC TME defined above. Although a co-culture of microbiome and escalation of biological complexity on the host side is possible, certain hallmarks of PDAC such as the strong desmoplastic reaction and the organoid bias towards a classical subtype (84) would be challenging to fully recapitulate PDAC complexity at the experimental level.

Discussion and future prospects: Towards a microbiome-targeted precision medicine

Current research in the personalized medicine field promises new hope for developing new tools for early diagnosis and for improving treatment of this deadly disease. Along these lines, our ever-expanding understanding of PDAC-SC and the interplay between intratumoral microbiome and oncogenes in all aspects of PDAC is promising. We now know that PDAC-SC play a fundamental role in the initiation and development of PDAC, and these cells are largely responsible for the aggressive, chemoresistant and metastatic nature of this cancer (26, 37, 85) (32, 43, 74). They are known to be dependent on niche factors (28–30). Thus, understanding the communication network that exists within the TME, including the PDAC-SC niche, are not only important for understanding PDAC pathogenesis, but may also be relevant at the level of resistance to conventional therapies and cellular plasticity.

As outlined above, evidence supporting a tumor-promoting role of an altered host microbiome at different sites is accumulating (34, 53, 71). This altered diversity may be a consequence of tumorigenesis, as the evolution of an inflammatory tumor microenvironment might promote bacterial translocation from the gut into the pancreas (25, 34, 56). Considering all the above evidence it is reasonable to speculate that the interplay between the intratumoral microbiota and oncogenic mutations promotes a specific PDAC-SC niche thereby impacting the tumor progression, chemoresistance and patient prognosis.

This interplay seems to be important in other gastrointestinal malignancies such as gastric cancer (86), esophageal cancer (87, 88). In this line, 26 microbial markers were proposed as early detection biomarkers to discriminate adenoma from colorectal cancer (89), and 30 microbial markers were identified and validated as diagnosis biomarkers in cohorts of individuals with early hepatocellular carcinoma and healthy controls (90).

In the case of PDAC, an improvement in treatment response due to the modulation of the patient's microbiome is already proposed in preclinical studies. Some even demonstrate a potential modulation of PDAC intratumoral microbiota with specific antibiotics overcoming gemcitabine and immunotherapy resistance in mouse models (57). In this regard, clinical trials focusing on compiling 16S rRNA profiles of PDAC patient samples and modulating microbiota are on the rise (based on http://clinicaltrials.gov/)

There is mounting evidence that patient microbiome composition can be used as a biomarker for disease progression as well as a druggable target to increase therapeutic efficacy of PDAC treatment. Therefore microbiome modulating strategies targeting the microbiome-dependent PDAC-SC niche would increase therapeutic responses and survival of PDAC patients paving the way towards the cure of this deadly disease.

Author contributions

Conceptualization and methodology: CMP-R and CB. Writing—original draft preparation, CMP-R and CB. Writing—review and editing, CMP-R, TR-T, LI-G, EC-B, JS-S, RM-S and CB. Supervision: CB. Project administration and funding acquisition: CB. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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