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Tumorigenesis and axons regulation for the pancreatic cancer: A mathematical approach

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ABSTRACT

The nervous system is today recognized to play an important role in the development of cancer. Indeed, neurons extend long processes (axons) that grow and infiltrate tumors in order to regulate the progression of the disease in a positive or negative way, depending on the type of neuron considered. Mathematical modeling of this biological process allows to formalize the nerve–tumor interactions and to test hypotheses in silico to better understand this phenomenon. In this work, we introduce a system of differential equations modeling the progression of pancreatic ductal adenocarcinoma (PDAC) coupled with associated changes in axonal innervation. The study of the asymptotic behavior of the model confirms the experimental observations that PDAC development is correlated with the type and densities of axons in the tissue. We study then the identifiability and the sensitivity of the model parameters. The identifiability analysis informs on the adequacy between the parameters of the model and the experimental data and the sensitivity analysis on the most contributing factors on the development of cancer. It leads to significant insights on the main neural checkpoints and mechanisms controlling the progression of pancreatic cancer. Finally, we give an example of a simulation of the effects of partial or complete denervation that sheds light on complex correlation between the healthy, pre-cancerous and cancerous cell densities and axons with opposite functions.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death in men and women. Late detection of this cancer, due to the near absence of symptoms in the early stages, is associated with a poor prognosis and an overall 5-year survival rate of less than 5% (Bengtsson et al., 2020). In recent decades, the impact of the microenvironment on tumor progression has become widely recognized, which has led to the development of new therapies such as immunotherapies. More recently, it has been shown that fibers of the nervous system infiltrate the tumor microenvironment where they also participate in the regulation of cancer development and progression (Guillot et al., 2022). It is therefore relevant to study the neurobiology of cancers through mathematical modeling in order to better understand the responses of cancer cells to innervation and predict at long term the effects of therapies targeting neuron-tumor interactions.

Mathematical modeling of the impact of the microenvironment on tumor progression has been widely investigated for many types of cancers. In the case of pancreatic cancer, a model of the interplay between the immune system and tumor progression has been proposed (Louzoun et al., 2014). As far as we know, mathematical modeling of the neural regulation of tumor progression has only be performed for prostate cancer (Lolas et al., 2016). This model confirmed experimental observations that a tumor is able to recruit nerves that, in turn, promote tumor development and metastatic spread. However, this initial model did not take into account the full functional diversity of neurons of the peripheral nervous system (PNS) and in particular their potential tumor suppressive effect discovered more recently in PDAC. To our knowledge, no mathematical model integrating the antitumor and pro-tumor activities of the PNS currently exists.

In this article, we developed an ordinary differential equation (ODE) model that describes and simulates the relationship between the PNS and pancreatic cancer development. The model is based on and calibrated with experimental data obtained from a genetically engineered mouse model of PDAC, in which the innervation of early pre-cancerous lesions and cancer have been characterized by three-dimensional (3D) histology (c.f Guillot et al., 2022). This model aims to investigate how dynamic changes in the neuronal composition of the microenvironment influence tumor progression.

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The paper is organized as follows. In Section 2, we review the biological background behind the mechanisms of the PDAC progression. We introduce the mathematical model and detail the assumptions made. In Section 3, we study the mathematical properties of the model. We prove its well-posedness and the convergence toward the pathological equilibrium under some assumptions. We extract some exponential convergence estimators which allow us to reduce the system and performed the asymptotic analysis on the limit system. In Section 4, we study the identifiability of the parameters when the model is confronted to the experimental data. We perform a sensitivity analysis which sheds lights on the effect of the axons on the PDAC progression.

2. Modeling the evolution of cell populations and axons

2.1. Biological background

The PNS is a vast network of nerves and ganglia that connect the brain to the other organs of the body. It consists of both afferent (sensory) nerves and efferent (motor) nerves that carry information in and out of the brain, respectively. While essential for internal body communication and proper regulation of physiological functions, the PNS also plays a newly-identified and pivotal role in the control of tumorigenesis. For example, denervation experiments in animal models of prostate and gastric cancers demonstrated a role of the visceral efferent motor system (also known as the autonomic nervous system) in promoting tumor progression and metastasis (Magnon et al., 2013; Zhao et al., 2014). These findings have led to the emerging concept of “nerve dependence in tumorigenesis” and a growing interest in repositioning inhibitors of nerve signaling for cancer treatment (Boilly et al., 2017; Zahalka and Frenette, 2020).

The bifunctional role of the PNS in pancreatic tumorigenesis. The impact of the PNS varies considerably depending on the tumor site. This has been highlighted by studies in animal models of PDAC. Indeed, and in contrast to its promoting role in prostate cancer, the autonomic nervous system has appeared to exert tumor suppressive effects in PDAC. The autonomic nervous system is divided functionally and anatomically into the sympathetic and parasympathetic nervous systems, which work together synergistically to regulate pancreatic functions (Love et al., 2007). In PDAC, several studies reported that transection of the vagus nerve, which provides parasympathetic inputs to the pancreas, promotes pancreatic cancer progression (Partecke et al., 2017; Renz et al., 2018). A similar acceleration of PDAC development and increased metastasis have been reported after selective depletion of pancreatic sympathetic innervation (Guillot et al., 2022), further supporting a protective function of the autonomic nervous system in this type of cancer. Conversely, the pro-tumoral influence of the PNS on PDAC is exerted by sensory neurons, whose selective ablation or functional silencing slows tumor progression and improves survival (Bai et al., 2011; Saloman et al., 2016; Sinha et al., 2017). Finally, when both sympathetic and sensory innervation of the pancreas are diminished (Guillot et al., 2022), this leads to an acceleration of PDAC development, suggesting a preponderant influence of the autonomic nervous system during tumor initiation. Thus, taking into account the functional specializations of nerve subtypes and their integration is crucial for understanding and predicting the trajectory of innervated tumors.

Neuroplastic changes associated with tumorigenesis. The PNS control over tumorigenesis is based on its ability to innervate developing tumors and release neurotransmitters, or other factors, in the cancer cell environment. Precise mapping of pancreatic tissue innervation and its evolution during PDAC development has been performed on histological sections and more recently using 3D light-sheet fluorescence microscopy (LSFM) on murine and human pancreas (Chien et al., 2019; Makhmoutova and Caicedo, 2021). The results revealed striking differences in the autonomic and sensory innervation patterns of the healthy pancreas, with a dense meshwork of autonomic nerve fibers (both sympathetic and parasympathetic) throughout the exocrine pancreas, from which PDAC arises, and an absence of sensory fibers in these same regions, the latter residing along the arteries and innervating the pancreatic islets (Guillot et al., 2022; Lindsay et al., 2006).

An initiating event for the development of PDAC is the transdifferentiation of acinar cells (the functional unit of the exocrine pancreas) into progenitor-like cells with ductal characteristics, a process called acinar-to-ductal metaplasia (ADM). ADM can progress to form preinvasive pancreatic intraepithelial neoplasia (PanIN) and eventually pancreatic cancer (Storz, 2017). A substantial innervation of the early pancreatic lesions by autonomic axons has been reported, with PanINs appearing as hotspots of sympathetic hyperinnervation (Guillot et al., 2022). While some sensory fibers can also be detected around PanINs, their density remains relatively low compared to autonomic fibers (Sinha et al., 2017; Stopczynski et al., 2014). In invasive PDAC tumors, however, this picture is completely reversed: a high density of sensory fibers deeply infiltrates the center of the tumors, while a moderate sympathetic innervation limited to the peripheral regions of the tumors was reported (Ceyhan et al., 2009; Guillot et al., 2022). In conclusion, the data revealed stage-specific remodeling of PNS networks during tumorigenesis that may have an important function by shifting an initially protective neural environment (autonomic > sensory) into a milieu favorable for cancer cell growth (sensory > autonomic).

2.2. Mathematical model

We will focus on the pancreas as the main domain of our model, thus including both the pancreatic cells and the nerve fibers (or axons). In this model, the variables for pancreatic cells densities are denoted \( Q_i \) for \( i \in \{ 0, 1, 2, 3 \} \), to take into account normal exocrine cells of the pancreas with \( Acini \), PanIN \( Q_0 \), and the different steps of tumorigenesis \( ADM Q_1 \), PanIN \( Q_1 \) and PDAC \( Q_2 \). The variables corresponding to PNS axons are denoted \( A_1 \), \( A_2 \), \( A_3 \) is the variation of the density of autonomic axons with respect to its equilibrium at initial state (denoted \( A_1^0 \)). The model does not distinguish between sympathetic and parasympathetic axons since they both have the same pro-tumoral function. Hence, we consider the following

\[
A_1 = \text{density of autonomic axons} - \text{initial equilibrium density.}
\]

This formalism allows \( A_1 \) to take negative values. One can justify it by the fact that neuroplastic changes of autonomic axons are non-monotonous: the density increases in PanIN and decreases in PDAC compared to the initial equilibrium density, i.e., the density in acini (Ceyhan et al., 2009; Guillot et al., 2022). Finally, \( A_2 \) is the density of sensory axons.

We propose multi-compartmental model in which the growth of the PNS is coupled to the transfer from acini to PDAC, and to the proliferation of pre-cancerous (PanIN) and cancerous (PDAC) cells. A schematic of the model, showing the variables and their interactions, can be found in Fig. 1.

Transfer between compartments. We consider that Acini progress in ADM with a transfer rate \( a_0 \). The transfer term is up-regulated by the presence of PanIN and PDAC through a Michaelis–Menten term with maximal amplitude \( \delta_0 \) and 1 as the Michaelis constant. This assumption leads to the following interpretation: the PanIN and the PDAC are able to self-promote and consequently decrease the density of Acini in the system. However the increasing influence of PanIN and PDAC on the transfer term is saturated. This transfer drives the dynamics of Acini and is described by the following equation:

\[
\frac{d Q_0(t)}{dt} = -\alpha_0 \left[ \frac{1 + \delta_0 Q_2(t) + Q_1(t)}{1 + Q_0(t) + Q_1(t)} \right] Q_0(t) + \text{transfer from Acini to ADM up regulated by the PanIN and PDAC}
\]

Next, ADM become PanIN with a transfer rate \( a_1 \). Moreover, the appearance of PanIN is positively correlated with a high density of autonomic
Axon growth dynamics. Neuronal plastic changes occur during the tumorigenesis and are closely linked to the presence of pre-cancerous and cancerous cells. Modeling innervation with a logistic law seems the natural way to describe this phenomenon if no spatial representation is taken into account. However, we do not take constant growth rates since the innervation is clearly induced by precancerous and cancerous cells.

We assume from the experimental data that the growth rate of autonomic axons is increased by ADM and PanIN, whereas PDAC has an opposite effect. The coefficients $\alpha_1$, $\alpha_2$, and $\alpha_3$ are associated respectively to $Q_1$, $Q_2$ and $Q_3$ in the growth term in order to specify the effect of each cell on the innervation. Also, the PanIN and PDAC cells promote sensory axon growth in a similar way with coefficients $\beta_1$ and $\beta_2$. One can reasonably consider that growth in a biological phenomenon is saturated because of various biophysical constraints. We introduced the threshold $\tau_{Ax}$ which is an upper bound for the density of sensory axons in the system. Similarly, we denote $\tau_{Ay}$ the threshold on autonomic axons. Note that $A_1$ is a difference quantity and this quantity is non-monotonous throughout the PDAC development process. Hence, we model the dynamic of $A_1$ by a modified logistic growth where the proliferation term is negative.

\[
\frac{d}{dt} Q_1(t) = \gamma_1 Q_1(t) \left( 1 - \frac{Q_1(0+Q_1(t))}{\tau_1} + \frac{A_1(t)}{\tau_{Ax}} + \frac{A_2(t)}{\tau_{Ay}} \right) \\
+ \kappa_1 \left[ 1 - \beta_1 A_1(t) \rho(A_1(t)) \right] Q_1(t) \\
- \kappa_2 \left[ 1 - \beta_2 A_1(t) \rho(A_1(t)) + \beta_2 A_2(t) \right] Q_2(t) \\
\text{proliferation regulated by the axons}
\]

\[
\frac{d}{dt} Q_2(t) = \gamma_2 Q_2(t) \left( 1 - \frac{Q_2(0+Q_2(t))}{\tau_2} + \frac{A_1(t)}{\tau_{Ax}} + \frac{A_2(t)}{\tau_{Ay}} \right) \\
+ \kappa_1 \left[ 1 - \beta_1 A_1(t) \rho(A_1(t)) \right] Q_1(t) \\
- \kappa_2 \left[ 1 - \beta_2 A_1(t) \rho(A_1(t)) + \beta_2 A_2(t) \right] Q_2(t) \\
\text{proliferation regulated by the axons}
\]

\[
\frac{d}{dt} Q_3(t) = \gamma_3 Q_3(t) \left( 1 - \frac{Q_3(0+Q_3(t))}{\tau_3} + \frac{A_1(t)}{\tau_{Ax}} + \frac{A_2(t)}{\tau_{Ay}} \right) \\
+ \kappa_2 \left[ 1 - \beta_2 A_1(t) \rho(A_1(t)) + \beta_2 A_2(t) \right] Q_2(t)
\]

Proliferation terms. We model cell proliferation in PanIN and PDAC by adding a logistic-like growth term for $Q_1$, $Q_2$, and $Q_3$ of respective rates $\gamma_1$, $\gamma_2$, and $\gamma_3$. The saturation term $\kappa_s$ is applied to the total density of proliferating cells which corresponds to $Q_1 + Q_2 + Q_3$. We model the PNS growth effect by incorporating the axons in the logistic law. We assume that the sensory axons promote the self-renewing growth of (pre)cancerous cells until the population attains a certain threshold. Furthermore, we assume that the autonomic axons have a reduced effect on this growth term. When the autonomic axons density is below its initial equilibrium state $A_1^{eq}$, the growth of (pre)cancerous cells is promoted. Once the density is lower than its equilibrium state, the cancerous cells still proliferate but attain a lower carrying capacity.
describe by the following equations

$$\frac{d}{dt} A_i(t) = (a_i Q_1(t) + a_i Q_2(t) - a_i Q_3(t)) \left(1 + \frac{A_i(t)}{r_{A_i}}\right) \left(1 - \frac{A_i(t)}{r_{A_i}}\right)$$

stimulus effect from ADM and PanIN and inhibiting effect from PDAC

logistic-like growth

(5)

$$\frac{d}{dt} A_2(t) = (\delta_2 Q_2(t) + \delta_2 Q_3(t)) A_2(t) \left(1 - \frac{A_2(t)}{r_{A_2}}\right)$$

stimulus effect from PanIN and PDAC

logistic growth

(6)

A priori conditions and assumptions on parameters. The interaction between cells and axons and the transition between cell populations are modeled by a dynamical system driven by the set of parameters \(\{x, \delta, \beta, \tau, \gamma, a\}\). In order to sum up, we recall that the transition rates are denoted by \(x\), the saturation rates by \(\tau\), the growth rates by \(\gamma\). The parameters \(\beta\) and \(\delta\) appear in the transition terms. The \(\beta\) parameters are coefficients which translate the inhibiting effect on the transition rates whereas the \(\delta\) parameters translate a stimulating effect on the transition rates. The last category of parameters is assimilated to the growth term of the axons. The parameters \(a\) and \(\bar{a}\) are associated to the impact of the cell populations on the growth rate of axons. All these parameters are assumed to be non-negative.

In the following, we assume the hypotheses:

**Hypothesis 1.** the transfer terms cannot become negative, it implies the following sufficient conditions:

$$1 > \beta_1 r_{A_1}, \quad 1 > \beta_2 r_{A_2}. \quad (H1)$$

**Hypothesis 2.** the growth rate of the cells in the logistic law gives rise to a competition between the PanIN cells and the PDAC cells for the same resource. However, the PDAC cells are considered to be dominant in the system and in the reality. It is translated by the following order relation on the parameters:

$$r_2 < r_3. \quad (H2)$$

**Hypothesis 3.** the autonomic axons \(A_i\) have a mixed effect on the proliferation term of PanIN and PDAC: it can either increase or decrease the resource in the logistic law. It is therefore unrealistic to consider that this mixed effect is the one that governs the dynamics of growth. This implies that the proliferation term cannot be of negative sign in the Eqs. (3) and (4). The following assumptions is assumed:

$$\tau_{A_1} < r_{A_1}. \quad (H3)$$

**Initial conditions.** We assume that at time 0, there are only Acini, autonomic axons and a very little amount of sensory axons. Hence, we have the following initial conditions:

$$Q_1(0) > 0, \quad Q_1(0) = Q_2(0) = Q_3(0) = 0, \quad A_i(0) = 0, \quad 0 < A_2(0) \ll 1.$$

(7)

Eqs. (1)–(6) form a non-linear dynamical system. The mathematical analysis of the system, such as the well-posedness, the positivity and the long-term behavior, assesses theoretically the legitimacy of modeling choices and improves the understanding of the interaction between axons and cancer.

3. Main results on the mathematical model

For the sake of clarity, we introduce an abstract formulation of the Eqs. (1)–(6). The main change is that the transition terms are now represented by the nonnegative functions \(f_i\) for \(i = 0, 1, 2,\) Hence, we obtain the following system:

$$\frac{d}{dt} Q_0 = -f_0(Q_0, Q_1)$$

$$\frac{d}{dt} Q_1 = f_0(Q_0, Q_3) - f_1(A_1) Q_1$$

$$\frac{d}{dt} Q_2 = f_2(Q_0, Q_1) - f_1(A_1) Q_2$$

$$\frac{d}{dt} Q_3 = f_3(Q_0, Q_3) + f_1(A_1) Q_3 - f_2(A_1, A_2) Q_2$$

$$\frac{d}{dt} A_1 = (\alpha_1 Q_1 + \alpha_2 Q_2 + \delta_2 Q_3) A_1 \left(1 - \frac{A_1}{r_{A_1}}\right)$$

$$\frac{d}{dt} A_2 = (\delta_2 Q_2 + \delta_2 Q_3) A_2 \left(1 - \frac{A_2}{r_{A_2}}\right)$$

(8)

One assumption of our model is that the transfer from healthy to cancerous cells is not reversible (H1). In this section, we further assume that the transfer is upper bounded (i.e., the progression from healthy to cancerous cells cannot be instantaneous). Therefore, this assumption can be conveniently phrased in terms of the above transition rates as:

$$\forall (x, y) \in \mathbb{R}^2 \quad \exists M_0 > m_0 > 0 \implies m_0 \leq f_0(x, y) \leq M_0,$$

$$\exists L_0 > 0 \quad \text{such that} \quad \forall u \in \mathbb{R}^2, \forall v \in \mathbb{R}^2 \quad \|f_0(u) - f_0(v)\| \leq L_0\|u - v\|. \quad (9)$$

and

$$\forall x \in \mathbb{R} \quad \exists M_1 > m_1 > 0 \implies m_1 \leq f_1(x) \leq M_1,$$

$$\exists L_1 > 0 \quad \text{such that} \quad \forall u, v \in \mathbb{R}^2 \quad \|f_1(u) - f_1(v)\| \leq L_1\|u - v\|. \quad (10)$$

and

$$\forall (x, y) \in \mathbb{R}^2 \quad \exists M_2 > m_2 > 0 \implies m_2 \leq f_2(x, y) \leq M_2,$$

$$\exists L_2 > 0 \quad \text{such that} \quad \forall u, v \in \mathbb{R}^2 \quad \|f_2(u) - f_2(v)\| \leq L_2\|u - v\|. \quad (11)$$

The first step in understanding the interactions between axons and the cancer progression is to establish properties of the model such as positiveness, well-posedness, etc. and to study its asymptotic behavior. The proofs of this section’s results are postponed in Appendix A.

3.1. Well-posedness

In the following section, we establish preliminary results on the model. Besides the well-posedness and the global existence of the solution, the cells densities are nonnegative. Moreover, the healthy cells densities (Acini and ADM) vanish at equilibrium. These results comfort the modeling since the simplistic but realistic outcome of the pancreatic cancer is the proliferation PDAC cells in the pancreas to the detriment of healthy cells.

**Theorem 1 (Well-Posedness).** Suppose the assumptions (9), (10) and (11) hold. Consider \(X = (Q_0, Q_1, Q_2, Q_3, A_1, A_2)\) and \(X^0 = (Q_0(0), Q_1(0), Q_2(0), Q_3(0), A_1(0), A_2(0))\) such that

$$Q_i(0) \geq 0, \quad \text{for} \quad i = 0, 1, 2, 3,$$

and

$$-\tau_{A_1} \leq A_1(0) \leq \tau_{A_1} \quad \text{and} \quad 0 \leq A_2(0) \leq \tau_{A_2}.$$

Then there exists a unique global solution for the system (8) with the initial condition \(X^0\) on \(\mathbb{R}_+\), moreover the following properties hold:

- **Nonnegativity** \(\forall t \in \mathbb{R}_+, Q_i(t) \geq 0, \quad \text{for} \quad i = 0, 1, 2, 3,\)

- **Boundedness of the axons** \(\forall t \in \mathbb{R}_+, -\tau_{A_1} \leq A_1(t) \leq \tau_{A_1} \quad \text{and} \quad 0 \leq A_2(t) \leq \tau_{A_2}.\)
3.2. Asymptotic behavior

In this section, the long time behavior of the solution of (8) is given by the following theorem.

Theorem 2 (Convergence Toward the Pathological Steady State). Suppose the assumptions (9), (10) and (11) hold. Assume that the initial conditions of (8) are such that (7) is verified. Assume that (H2) and (H3) hold. Then

\[
X(t) = \left( Q_0(t), Q_1(t), Q_2(t), Q_3(t), A_1(t), A_2(t) \right)
\rightarrow \left( 0, 0, 0, \frac{\tau_C C(-\tau_{A_1})}{\tau_{C} + \tau_{A_1}}, -\tau_A, \tau_{A_2} \right)
\text{ for } t \rightarrow +\infty
\]

where \( \frac{\tau_C C(-\tau_{A_1})}{\tau_{C} + \tau_{A_1}} = 1 - \frac{\tau_{A_1}}{\tau_C} + \frac{\tau_{A_2}}{\tau_{A_1}} \).

Sketch of the proof: The proof of Theorem 1 is detailed in Appendix A.1. It relies on the proof of the existence and uniqueness of a maximal solution. This result can be found in Proposition 1. Moreover, this result implies that the cell densities of the Acini \( Q_1 \) and the ADM \( Q_2 \) decay exponentially fast toward 0 (c.f. Proposition 2). Hence, these decays show that the time evolution of the cell densities of the PanIN \( Q_3 \) and the PDAC \( Q_4 \) are bounded and that there exists a unique global solution (c.f Proposition 2).

4. Calibration of the system and results

Next, we calibrated the model with biological information captured in the data. The calibration seems to be in general a subjective approach. In our case, the large number of input parameters renders it challenging and highly time-consuming. We use an optimization method in order to reduce the user intervention and obtain a more objective selection of parameters.

We first introduce and study the biological information aggregated in the data. Then, we construct an objective function that integrates the biological assumptions and the experimental data. We study the identifiability of the parameters in regard to this objective function.

Finally, we obtain sets of parameters which calibrate reasonably well the model and we discuss the sensitivity of these parameters.

4.1. Biological assumptions and observation

The observation aggregates experimental data and empirical knowledge such as experimentally validated hypotheses (time of appearances, etc.). We gather all the biological information available on the process and formalize into the vector of observation denoted \( y^o \) and the vector of chronomolecular parameters \( (t_i)_{i=1,5} \).

4.1.1. Experimental data 1

The observation used as referential in order to calibrate the model is represented in Fig. 2. Data were obtained from the KIC (LSL-KrasG12D/+; Cdkn2a (Ink4a/Arf)lox/lox; Pdx1-Cre) transgenic mouse model of PDAC and describe the percentage of acinar tissue, ADM, PanIN and PDAC in histological sections through the pancreas of a 6.5 weeks-old mouse (45 days). Quantification data have been published and are available in the source data file of Guillot et al. (2022). Because of the variability between samples, we choose to aggregate all the information contained in each section of the same mouse. We consider the observation as the proportion of each compartment averaged over all samples of the same mouse and denote these experimental data \( y^o_1 \) for the Acini, \( y^o_2 \) for the ADM, \( y^o_3 \) for the PanIN and \( y^o_4 \) for the PDAC. In addition, the time of tissue harvest is 45 days and is denoted \( t_f \) in the following.

Remark 2. One can reasonably object that considering a larger number of tissue sections is consistent with the fact that the domain in the mathematical model is the whole pancreas and its immediate environment. However, one of the purpose of this work is to be able to use human biopsies as data. In the case of human data, the number of biopsies is limited and it becomes interesting to develop generic models and methods that make up for the lack of information.

4.1.2. Experimental data 2

Data on axonal density were obtained by quantifying innervation in the pancreas of control and KIC mice. Whole pancreases were immunostained with antibodies specific to each PNS neuron subtype.

Sympathetic axons were immunolabeled with an antibody against tyrosine hydroxylase (TH), parasympathetic axons with an antibody against vesicular acetylcholine transporter (VACHT) and sensory fibers with an antibody against calcitonin gene-related peptide (CGRP). The tissues were then imaged using LSFM to allow 3D visualization of the neuronal networks. LSFM images were processed with Imaris software. Regions of Interest (ROIs: Acini, ADM, PanIN or PDAC) were segmented based on the autofluorescence signal of the tissue and their volumes were measured. Axonal networks were manually reconstructed using the Imaris "Filament tracer" tool. The "dendrite length sum" was collected on the autofluorescence signal of the tissue and their volumes were measured. Axonal length sum (nm) / volume of ROI (\( \mu m^3 \)). The full protocol and the quantification data for the sympathetic axons are available in the source data file of Guillot et al. (2022). The quantification data for parasympathetic and sensory fibers are performed using the same protocol. To obtained
the density of autonomic axon, we sum the density of sympathetic and parasympathetic axons in each ROI. Experimental data were measured at day 45 and denoted $y'_1$ for the autonomic axons, $y'_6$ for the sensory axons and $A^q_{1}$ the density of autonomic axons of the control mouse. The experimental data expressed in nm(μm)$^{-3}$ are as follows :

\[
y'_1 = 0.1077, \quad y'_6 = 0.1468, \quad A^q_{1} = 0.0099.
\]

4.1.3. Biological assumptions on the chronological process

Initial conditions: there are only healthy cells (Acini) at initial time and there is a negligible amount of sensory axons in the pancreas and a small amount of autonomic axons at initial time. These conditions are formalized by (7). In addition, the neuroplastic changes and the cancer progression are relatively negligible at early stages (before two weeks of age in the KIC model). We denote $t_2$ the parameter (in days) corresponding to the initial time for the model simulations. Without loss of generality, we consider $t_2 = 10$ days.

Chronological appearances: Based on the previous characterization of the KIC model (Aguirre et al., 2003 and our personal observations), the first appearance of ADM is around time $t_1 = 17$ days, of PanIN around time $t_2 = 21$ days and PDAC around time $t_3 = 35$ days. The density of autonomic axons increases in ADM regions, peaks in PanIN and decreases in PDAC, while sensory axons are rarely observed in PanIN, but have a high density in PDAC. We therefore empirically set the time of appearance of $A_1$ and $A_2$ at $t_4 = 18$ days and $t_5 = 30$ days, respectively.

4.2. Parameters calibration with an optimization procedure

The dynamics of the system depends very strongly on the choice of parameters. This choice is based on the calibration of the model. In other words, it is first necessary to quantify the distance between the outputs of the model and the biological data and then to find the parameters that minimize this distance in an objective way. That is the reason why, testing the goodness of fit through an optimization procedure impose itself as a rigorous method. The following section describes a data-driven process which minimizes the deviation between the observation and the model and which gives an objective calibration of the parameters.

4.2.1. Objective function

The measurement of this deviation is made possible by the objective function or also called the cost function which is denoted as $C$. The inputs of $C$ are the parameters of (1)–(6) (see Table 2) denoted as the vector $\theta$. This cost function integrates the biological assumptions and the comparison to the experimental data. The further away from the experimental data the trajectories are, the higher the cost function is. Hence, finding the parameters which minimize the cost function is equivalent to the calibration of the model. We recall that $y^* \in \mathcal{K}$ where $\mathcal{K}$ is a compact set in $\mathbb{R}^s$. The vectors of parameters $\theta^*$ which minimize the function $C$ give the optimal calibration of the system where the biological constraints and hypothesis are taken into accounts. The cost function is described by the following equation :

\[
C(\theta) = \sum_{k=1}^{3} \left( a_k \int_{t_{k-3}}^{t_k} (Q_k(s) - \gamma^*_s)^2 ds + b_k \int_{t_{k-3}}^{t_k} (A_k(s) - \gamma^*_s)^2 ds \right)
\]

\[
+ \sum_{k=1}^{4} (b_k \int_{t_{k-3}}^{t_k} (Q_k(s))^2 ds + b_k \int_{t_{k-3}}^{t_k} (A_k(s))^2 ds + b_k \int_{t_{k-3}}^{t_k} (A_k(s))^2 ds)
\]

where $C(\theta)$ is the function defined as above and $Q_k(s)$ and $A_k(s)$ are the simulated and experimental data, respectively.

The parameter $t_{f}$ corresponds to the time in days of data extraction from in vivo experimentations (see Fig. 2), hence $t_{f} = 45$. The definition of (13) also includes the interval of days $[t_{j-3}; t_{j} + 3]$ and the parameters $(a_k)_{k=0, \ldots, 5}, (b_k)_{k=1, \ldots, 5}$. The time interval $[t_{j-3}; t_{j} + 3]$ allows us to use the $L^2$-norm squared to compare the simulated trajectories to the observation. However, it implies that the experimental data are supposed to be true on a six days interval around $t_{j}$. One can reasonably justify this assumptions by the fact that this non-local norm regularizes the observation from its inherent biological chaos. Chaos means that biological phenomenon, processes or experiments are extremely sensitive to small perturbations. In our case, it is translated by the variability of chronological appearances of phenomenon between mice. The $L^2$-norm smooths the variability in time in the objective function and is less sensitive to the observation variability.

Remark 3. We introduce $a_k$ and $b_k$ as normalization parameters in $C$. The objective function $C$ is a sum of positive terms where each of these terms have a contribution to the final cost. These contributions are individually linked to a specific part of the information on the biological process. However, there are discrepancies between the different parts, for instance, $y^*_2 \ll y^*_1$ (cf. Fig. 2) or $t_{j} \ll t_{j+1}$. Some data can be falsely assimilated as outliers. Hence, we consider the following :

\[
a_k = \frac{1}{6y^*_k} \quad b_k = f_k(y^*_k) = \frac{1}{t_{k} - t_{0}},
\]

where $f_k$ adjusts the coefficient $b_k$ in regard to $y^*_k$. Ultimately, the normalization coefficients make all contributions to the cost function equal.

4.3. Identifiability and sensitivity of the parameters

We study the identifiability of the model to understand the degree to which the parameters can be constrained to a unique value or a reasonable range of values given the data available. Since multiple model
parametrizations produce similar behavior (see Section 4.3.1), we use profile likelihood methods (cf. Kreutz et al., 2013; Roosa and Chowell, 2019) to refine the interpretation of the estimators (see Section 4.3.4).

4.3.1. Optimization challenges

The optimization problem leads to mathematical and numerical challenges. With such experimental data and such a large set of parameters, we cannot expect to calibrate the model uniquely. It leads to multiple local minimizers and convergence issues. We use the Covariance Matrix Adaptation Evolution Strategy (CMA-ES see Hansen 2006, Jastrebski and Arnold 2006) which is based on a derivative-free evolution algorithm in order to overcome the obstacles linked to the size of the optimization problem and to the non-trivial dependence of the cost function on the parameters. The results of the optimization procedure give a set of acceptable parameters. The evolution of cell populations can be simulated for each of these parameters’ vector (cf. Fig. 3). It is interesting to note that the trajectories in the numerical simulation show different behaviors for the same cost (see Fig. 3).

Recall that the expected asymptotic behavior corresponds to the depletion of all healthy and precancerous cells and the saturation of the cost function on the parameters. The results of the optimization procedure give a set of acceptable parameters. The evolution of cell populations can be simulated for each of these parameters’ vector (cf. Fig. 3). It is interesting to note that the trajectories in the numerical simulation show different behaviors for the same cost (see Fig. 3).

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problem in the literature and has beneficial properties like efficiency and consistency (Van der Vaart, 2000).

4.3.3. Profile likelihood to identify the parameters

The issue remains the large number of parameters and the "small" amount of data at our disposal. Therefore, the study of the identifiability of the parameters gives further knowledge about the fit between the model and the observation and helps to interpret the outputs given by the model. Since the optimization problem is linked to the maximization of the likelihood, the profile likelihood solves the identifiability problem (Murphy and Van der Vaart, 2000). This one-dimensional projection is performed to visually evaluate whether different values of the same parameter give similar outputs. We recall that the vector $\theta$ denotes the parameters of the model. Hence, using the objective function (16), the impact of the value of the specific parameter $\theta_j$ for fitting the model to the observation is assessed by the following profile likelihood:

$$P_j(\rho) = \min_{\hat{\rho} \in \mathbb{R}^{(i\neq j)}} \chi^2(\hat{\rho}),$$  \hspace{1cm} (17)$$

where the objective function (16) is evaluated as a function of the values $\rho^i$ taken by the parameter $\theta_j$ while all others parameters $\theta_{i\neq j}$ are reoptimized. This one-dimensional representation of the likelihood (17) can be geometrically interpreted in order to assess the identifiability of the parameter $\theta_j$ (Kreutz et al., 2013; Raue et al., 2009). For instance, a flat profile for $P_j$ corresponds to a structural non-identifiability for the parameter $\theta_j$. It implies that the parameter is non-unique for the minimization of the objective function. Eliminating the non-uniqueness requires more data or additional data on different quantities. If $P_j$ has a minimum but is flat on one side then the parameter $\theta_j$ is considered as a practically non-identifiable parameter. It implies that the data do not contain significant enough information about the parameter. The parameter value cannot be restricted to a precise value. Similarly, new experiments leading to additional data are required in order to rigorously estimate the parameters. However, if $P_j$ describes a curve with unique minimum in a realistic range of values then the parameters $\theta_j$ is identifiable. Also, if some knowledge is assumed on the experimental noise $\epsilon$, a finite confidence interval can be computed and gives an asymptotic validation of the identifiability of the parameter $\theta_j$ (see Murphy and Van der Vaart 2000, Kreutz et al. 2013, Raue et al. 2009).

An implementation of the profile likelihood has been performed where $\theta_j$ takes 20 distinct values denoted $\{\rho^i | i = 1 \ldots 20\}$ which cover its range (see Table 2). For each $\rho^i$, the minimization problem (17) has been solved numerically with 50 different initial conditions on the parameters $\theta_{i\neq j}$ in order to fully explore the optimization domain (see Appendix C). This Monte-Carlo approach allows to obtain several sets of admissible parameters for each $\rho^i$. It gives a qualitative criterium to validate the identifiability of a parameter: if the median of the costs $P_j$ admits a distinct minimum at $\rho^{i*}$ then we consider the parameter to be identifiable and its value to be $\rho^{i*}$. It is reasonable to consider as estimator the identifiable parameters $\rho^{i*}$, however these values are highly dependent on the choice of the discretization of the $\{\rho^i\}$. This uncertainty related to this choice is significantly reduced when we focus on the confidence range obtained through the procedure, i.e. the range where the identifiable parameters give reasonable results concerning the deviation between the observations and the model.

As a result, we clearly distinguished seven identifiable parameters in our model (see Fig. 4): the progression transfer rates $\pi_0$, $\pi_1$ and $\pi_2$, the proliferation rates of the PanIN and the PDAC respectively $\gamma_2$ and $\gamma_3$, the saturation term for the proliferation of the PanIN and PDAC $\tau_C$ and finally the amplitude of the Michaelis–Menten term describing the effect of the PanIN and the PDAC on the progression of the Acini into ADM $\delta_0$. These parameters and their estimated values are gathered in the Table 1.

The identifiability of these seven parameters is further verified by comparing the cost distribution of the objective function in two cases: when we fix the identifiable parameters and minimize on all the remaining parameters and when all the parameters are free during the optimization. In the first case, the cost distribution is located around 4 (close to the minimal value obtained in the numerical simulation). In the second case, the cost distribution is much more spread (see Fig. 10 in the Appendix). It confirms that the parameters are correctly estimated thanks to the data available since the distribution of fitting errors is concentrated on small values. Further details about the numerical methods are postponed in Appendix C. In the end, this method allows to obtain a data-driven identifiability criterion without making

![Fig. 4. Numerical results of the identifiability problem using the profile likelihood. Each plot corresponds to a specific identifiable parameter ($\pi_0, \pi_1, \pi_2, \gamma_2, \gamma_3, \tau_C, \delta_0$). The y-axis describes the values taken by the fixed parameter $\theta_j$. The x-axis denotes the corresponding fitting error defined in (17). The gray area represents 75% of the results between the first and last quartile (see Appendix C). The median (in blue) and the mean (in orange) for 50 iterations of the minimization problem show that each parameters admits a distinct minimum value under 10 for the fitting error.](image_url)
assumptions on the experimental noise. It also gives estimators and their confidence range (see Table 1) for the identifiable parameters of the model.

4.3.4. Mathematical and biological interpretations

What can we infer about pancreatic cancer progression from the biological data? This numerical study around the identifiability of the parameters of the model gives interesting insights about the experimental data and the biological processes behind it. First, we observed that the transfer rates from Acini to ADM $\tau_0$, ADM to PanIN $\tau_1$, and PanIN to PDAC $\tau_2$ are identifiable. Moreover, the estimates for the transfer rates respect the following order relation $\tau_2 > \tau_1$. It implies that the progression process appears to go faster at the late stage of the PDAC development. This is consistent with the rapid tumor development reported in the KIC mice used for model calibration.

We also obtain estimates for the proliferation rates of the PanIN $\gamma_2$ and the PDAC $\gamma_3$. It implies that the data combining the proportion of cell populations at 45 days and the knowledge of the first time of appearance of each cell population give a sufficient amount of knowledge in order to calibrate the speed of the proliferation mechanism in the model. However, the threshold parameters $\gamma_i$ is likely to be underestimated in regards to the data at our disposal. The value of this threshold is of a different order of magnitude compared to the other parameters. Therefore, its range of acceptable estimates is also wider compared to the other parameters and additional data are needed in order to compute a finer estimate.

What is the impact of the axons regulation on the tumor progression? A variance-based sensitivity analysis (see Sobol’ 1990, Sobol 2001) allowed us to investigate this issue. In order to perform this analysis, we restrict ourselves to the parameters linked to the effect of the axons on the cell population densities: the parameters in the transition rates $\beta_1$, $\beta_2$ and $\delta_2$, the parameters in the proliferation terms $\gamma_{A_1}$ and $\gamma_{A_2}$ (c.f. Table 2). The other parameters are fixed and are given by the last set of parameters in the Table 3. The choice of this specific set of parameters is based on qualitative considerations such as its associated cost given by (13), the fact that the associated trajectories are similar to the expected behavior (e.g. the decay of $A_1$, etc.). Hence, the inputs are these five parameters, which are denoted $\theta = \left(\beta_1, \beta_2, \delta_2, \gamma_{A_1}, \gamma_{A_2}\right)$.

Concerning the output of the sensitivity analysis, our focus is on the PDAC cells. We introduce the following indicator of the variation of the PDAC cells:

$$V = 1 - \frac{\int_{t_0}^{t_f} Q_3(s; \theta) ds}{\int_{t_0}^{t_f} Q_3(s) ds},$$

where $Q_3(s)$ denotes the PDACs density for the control parameters set (given by the whole last set of parameters in the Table 3), $Q_3(\cdot; \theta)$ denotes the PDAC cells density for the input parameters $\theta$, $t_0 = 10$ and $t_f = 70$ correspond to the initial time and the finite time for the model simulations. The interpretation of the output $V$ in (18) is the following:

- if $|V| \leq \epsilon$ for $\epsilon$ arbitrary small, then axons have almost no effect on the appearance of PDACs between days $t_0$ and $t_f$.
- If $V > \epsilon$, the inputs in $\theta$ have an inhibiting effect on the PDACs between days $t_0$ and $t_f$.

<table>
<thead>
<tr>
<th>$\tau_0$</th>
<th>$\tau_1$</th>
<th>$\tau_2$</th>
<th>$\gamma_2$</th>
<th>$\gamma_3$</th>
<th>$\delta_2$</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
<th>$\delta_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0025</td>
<td>0.00942</td>
<td>0.176</td>
<td>0.229</td>
<td>0.31</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
| $(10^{-4}, 10^{-5})$ | $(10^{-5}, 10^{-6})$ | $(10^{-6}, 0.5)$ | $(10^{-5}, 0.3)$ | $(0.2, 0.4)$ | $(50, 200)$ |$(3, 5)$

Fig. 5. Indices of the sensitivity analysis of the parameters linked to the axons on the PDAC cells. The color blue correspond to the first-order-effect indices (SI) of the Sobol sensitivity analysis and the color orange correspond to the total-effect indices (ST). The black segment corresponds to the confidence interval for the associated sensitivity index.
• Conversely, if \( V < -\epsilon \), the inputs in \( \delta \) have a positive impact on the PDACs between days 10 and 70 which can also be interpreted as a pro-tumoral effect of the axons.

The results of the variance-based sensitivity analysis are given by the first-order indices and the total-effect indices respectively \( S_1 \) an \( ST \) in the Fig. 5 (see Saltelli et al. 2000). Both types of index measure the contribution of the effect \( \theta_i \) to the output variance. However, the first-order index measure the effect of varying \( \theta_i \) alone and is averaged over the variations of the other input parameters. Whereas, the total-effect index gives the contribution of \( \delta_i \) and its interactions with any other inputs or tuple of inputs. This approach is also called the global sensitivity analysis because it also measures the sensitivity of any tuple of inputs.

Ultimately, the following conclusions can be drawn from the sensitivity analysis (see Fig. 5).

• The two main contributors to the variability of the PDACs are the parameters \( \beta_1 \) and \( r_C^2 \). Both parameters have an inhibiting effect on the PDACs when their values are growing. This means that PDAC progression is mainly regulated by the effect of autonomic axons on the ADM to PanIN transition and by the effect of sensory axons on cell proliferation.

• By definition, we have that \( ST(\theta_i) \geq S_1(\theta_i) \) and the equality holds when the model is additive. One interesting remark is that the first-order index and the total-effect index of \( r_C^2 \) are almost equivalent. This implies that the contribution of cross-effects between \( r_C^2 \) and the other parameters is small. Thus, PDAC proliferation is under the near exclusive control of sensory axons, with other axonal populations having little or no contribution.

• The first-order indices of \( \beta_2, \delta_2 \), and \( r_C^2 \) are significantly smaller than the indices of the two other parameters. This implies that the variation of these parameters, taken one by one, has a relatively negligible effect on the variations of PDACs. Moreover, since the total-effect index of \( \beta_2 \) is small, this implies that the inhibitory impact of autonomous axons on the transfer of PanIN cells to PDACs does not have much impact on the overall amount of PDACs in the model.

• Concerning \( \delta_2 \) and \( r_C^2 \), the discrepancy between the first-order indices and the total-effect indices indicates that the parameters still have an effect on the overall amount of PDACs. However, the significance of the effect is primarily seen through the interactions between one of these two parameters and the others (i.e., when measuring the impact of varying pairs or tuples of parameters simultaneously).

What is the impact of denervation on the tumor progression?

To address this issue, we consider the dynamics associated to the last first-order index measure the effect of varying \( \theta_i \) alone and is averaged over the variations of the other input parameters. Whereas, the total-effect index gives the contribution of \( \delta_i \) and its interactions with any other inputs or tuple of inputs. This approach is also called the global sensitivity analysis because it also measures the sensitivity of any tuple of inputs.

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5. Conclusion

In this paper, we develop an original model to investigate the role of peripheral axons in pancreatic cancer progression. The study of the calibration to the experimental data highlights the generality of the model and the fine analysis of the data informs on the underlying mechanisms. For example, the model recapitulates the drastic acceleration of tumorigenesis observed in KIC mice, and attributes it to the short latency in the transition from neoplasms to invasive cancer. Furthermore, our data highlight two major “neural checkpoints” in PDAC progression: an early phase during which progression is restrained by autonomic axons at the ADM-PanIN transition, and a later window of control of PDAC growth by the promoter action of sensory axons on cell proliferation. These results suggest that cancer-induced neuronal plasticity may promote a shift from a protective to a harmful role of the peripheral nervous system on pancreatic cancer. Finally, our model also allowed us to simulate the effect of partial or complete denervation of autonomic and/or sensory axons and to recapitulate the different results observed in tumor progression in experimental in vivo models. In particular, the acceleration of PDAC growth observed after joint denervation of pro- and anti-tumor fibers (i.e., complete autonomic and sensory denervation) underscores the predominant effect of the checkpoint by autonomic axons on subsequent tumor progression.

A first step for expansion and improvement is to investigate further the acquisition of the experimental data in order to obtain quantitative data reducing the predictive uncertainty of the model. As an example, additional measurements are needed to quantify more precisely the evolution of sensory and autonomic axon density over time. These measurements would be related to the speeds of axon density in the model. Moreover, these speeds aggregate into a coefficient various effects coming from different cell populations. This uncertainty can be reduced by studying and building an optimal experimental design in relation to the mathematical model.

The model could be further improved by considering the phenotype of the cell as a continuous variable. In particular, this approach leads to a coupled model with a partial differential equation and differential equations. This formalism would allow a more precise description of the tumor progression and the neuroplastic changes occurring during this process. In particular, this would allow the incorporation of neglected pre-malignant stages (e.g. PanIN 1, PanIN 2, PanIN 3, etc.) into the model and it would lead to a finer representation of the progression of pancreatic cancer in human patients.

An additional extension step would be to more accurately include other component of the tumor microenvironment (as example the immune system) in the modeling. However, the precision gained in the modeling automatically leads to an increase in the data required for...
calibration and an increase in the complexity of the predictions that can be made by this new model.

The present model of neural regulation of pancreatic cancer may have clinical applications. Indeed, the discovery of the role of neuronal signaling in pancreatic cancer progression has made therapeutic targeting of neuronal pathways a new area of clinical interest. For example, a randomized placebo-controlled trial showed a survival benefit of denervation in pancreatic cancer patients with high sensory nerve activity (Lillemoe et al., 1993). However, peripheral denervation is not permanent, and reinnervation and eventual tumor progression occur with time. Our mathematical model could serve as a basis to help determine the frequency, dosage and duration of temporary denervation treatments. In addition, it has been suggested that pharmacological inhibition of neural signaling may act synergistically with chemotherapy to inhibit pancreatic tumor growth (Renz et al., 2018). An extension of our mathematical model that include cell response to chemotherapy could in principle be used to identify neural drug delivery strategies and combination schedules with maximal inhibitory effect on PDAC.

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Appendix A. Mathematical results

This section is devoted to the proof of Theorems 1 and 2.

A.1. Properties of the system (8)

Proposition 1. Let \( T > 0 \) be arbitrary and consider \( X = (Q_0, Q_1, Q_2, Q_1, A_1, A_2) \) and \( X^0 = (Q_0(0), Q_1(0), Q_2(0), Q_1(0), A_1(0), A_2(0)) \) such that \( Q_i(0) \geq 0, \) for \( i = 0, 1, 2, 3 \) and

\[-r_{A_i} \leq A_1(0) \leq r_{A_i} \quad \text{and} \quad 0 \leq A_2(0) \leq r_{A_2}.\]

Moreover assume that

\[\forall (x, y) \in \mathbb{R}^2 \exists M_0 > m_0 > 0 \implies m_0 \leq f_0(x, y) \leq M_0,\]

\[\exists L_0 > 0 \quad \text{such that} \quad \forall u \in \mathbb{R}^2, \forall v \in \mathbb{R}^2 \quad \|f_0(u) - f_0(v)\| \leq L_0\|u - v\|,\]

\[\forall x \in \mathbb{R} \exists M_1 > m_1 > 0 \implies m_1 \leq f_1(x) \leq M_1,\]

\[\exists L_1 > 0 \quad \text{such that} \quad \forall (u, v) \in \mathbb{R}^2 \quad \|f_1(u) - f_1(v)\| \leq L_1\|u - v\|,\]

\[\forall (x, y) \in \mathbb{R}^2 \exists M_2 > m_2 > 0 \implies m_2 \leq f_2(x, y) \leq M_2,\]

\[\exists L_2 > 0 \quad \text{such that} \quad \forall u \in \mathbb{R}^2, \forall v \in \mathbb{R}^2 \quad \|f_2(u) - f_2(v)\| \leq L_2\|u - v\|,\]

Then there exists a unique maximal solution for the system (8) with the initial condition \( X^0 \) on \( I = [0, T). \) The following properties hold:

- **Nonnegativity** \( \forall t \in I \quad Q_i(t) \geq 0, \) for \( i = 0, 1, 2, 3, \)
- **Boundedness of the axons** \( \forall t \in I \quad -r_{A_i} \leq A_i(t) \leq r_{A_i} \quad \text{and} \quad 0 \leq A_2(t) \leq r_{A_2}. \)

CRediT authorship contribution statement

**Sophie Chauvet:** Investigation, Conceptualization, Writing – original draft, Writing – review & editing. **Florence Hubert:** Funding acquisition, Formal analysis, Visualization, Software, Conceptualization, Writing – original draft, Writing – review & editing. **Fanny Mann:** Investigation, Funding acquisition, Conceptualization, Writing – original draft, Writing – review & editing. **Mathieu Mezache:** Formal analysis, Visualization, Software, Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fig. 6. Numerical simulations of the time evolution of PDAC cells. The x-axis describes the time in day. Each curves corresponds to the simulation of the quantity \( Q_i \) in different cases: when there is no denervation (blue), when the effect of the autonomic axons is off (orange), when the effect of the sensory axons is off (red) and when the effect of the autonomic and sensory axons is off (green). The set of parameters for the simulation is given in the Table 3 (last set of parameters).
Proof. The well-posedness is a direct consequence of the Cauchy-Lipschitz theorem and the Lipschitz bound on $f$. Moreover, 
\[ \frac{d}{dt} Q_0(t) = - f(t)(Q_2(t), Q_3(t)) Q_0(t) = - \psi(t) Q_0(t) \]
for a positive function $\psi$, then $\{0\}$ is an invariant set for $\frac{d}{dt} Q_0$ and we have $Q_0(t) \geq 0$. Similarly, we obtain that 
\[ \forall t \in I - r_{A_1} \leq A_1(t) \leq r_{A_1} \text{ and } 0 \leq A_2(t) \leq r_{A_2}. \]
Then, using the boundedness of $f_0$ and $f_1$ and the nonnegativity of $Q_0$, we obtain 
\[ \frac{d}{dt} Q_1(t) \geq -M_1 Q_1(t). \]
Hence by Gronwall’s lemma, we have 
\[ \forall t > 0 \quad 0 \leq Q_1(t) e^{-M_1 t} \leq Q_1(t). \]
Similarly, using the boundedness of $f_1$ and the nonnegativity of $Q_1$, we obtain 
\[ \forall t > 0 \quad 0 \leq Q_1(t) e^{\phi_1(t)} \leq Q_1(t), \]
where 
\[ \psi(t) = \int_0^t \left( 1 - \frac{Q_2 + Q_3}{\tau_1} + \frac{A_1}{\tau_1} + \frac{A_2}{\tau_2} \right) - M_2 ds. \]
Again, using the boundedness of $f_2$ and the nonnegativity of $Q_2$, we obtain 
\[ \forall t > 0 \quad 0 \leq Q_2(t) e^{\phi_2(t)} \leq Q_2(t), \]
where 
\[ \psi(t) = \int_0^t \left( 1 - \frac{Q_2 + Q_3}{\tau_1} + \frac{A_1}{\tau_1} + \frac{A_2}{\tau_2} \right) ds. \]

**Proposition 2.** Suppose the same hypotheses as in Proposition 1, then the following properties hold: 

1. **Exponential decays** The solutions $Q_0$ and $Q_1$ decay exponentially fast toward 0 as $t$ tends to infinity. Moreover, 
\[ \forall t \in I - Q_0(t) e^{-M_0 t} \leq Q_0(t) \leq Q_0(0) e^{-m_0 t}, \]
and it exists a constant $C(Q_0(0), Q_1(0)) \geq 0$ such that 
\[ \forall t \in I - 0 \leq Q_1(t) \leq C(Q_0(0), Q_1(0)) e^{-(m_1 - \alpha_1) t}. \]

2. **Global existence** There exists a unique solution for the system (8) with initial condition $X_0$ on $\mathbb{R}_+$. 

**Proof.** 1. (Exponential decays) Using the Gronwall’s lemma, we get the following from the boundedness of $f_0$ 
\[ \forall t > 0 \quad Q_0(t) e^{-M_0 t} \leq Q_0(t) \leq Q_0(0) e^{-m_0 t}. \]
From the proof of nonnegativity, we obtain a lower bound on $Q_1$ which decays exponentially fast 
\[ \forall t > 0 \quad Q_1(t) e^{-M_1 t} \leq Q_1(t). \]
We are now interested in the upper bound of $Q_1$. Using the bound on $f_1$ and the Gronwall’s lemma, we obtain 
\[ \frac{d}{dt} Q_1(t) \leq M_0 e^{-m_0} Q_0(0) - m_1 Q_1(t), \]
\[ Q_1(t) \leq e^{-m_1 t} Q_1(0) + e^{-m_1 t} M_0 Q_0(0) \int_0^t e^{(m_1 - m_0) s} ds. \]
We first consider the case where $m_1 = m_0$, thus we get 
\[ Q_1(t) \leq e^{-m_1 t} Q_1(0) + e^{-m_1 t} M_0 Q_0(0), \]
\[ Q_1(t) \leq e^{-m_1 t} Q_1(0) + e^{-m_1 t} M_0 Q_0(0), \]
\[ Q_1(t) \leq (e^{-m_1 t} Q_1(0) + M_0 Q_0(0)) e^{-m_1 + \alpha_1} Q_1(t). \]
We finally get 
\[ Q_1(t) \leq \left[ Q_1(0) + M_0 Q_0(0) \right] e^{-(m_1 - \alpha_1) t}. \]
Let us now consider the case where $m_1 \neq m_0$, 
\[ Q_1(t) \leq e^{-m_1 t} Q_1(0) + e^{-m_1 t} M_0 Q_0(0) \frac{e^{m_2 t} - e^{-m_0 t}}{m_1 - m_0}, \]
\[ Q_1(t) \leq e^{-m_1 t} Q_1(0) + M_0 Q_0(0) \frac{e^{m_2 t} - e^{-m_0 t}}{m_1 - m_0}. \]
We denote $m = \min(m_0, m_1)$, we also obtain an exponential decay: 
\[ Q_1(t) \leq \left[ e^{-m_1 t} - m_0 \right] Q_0(0) + M_0 Q_0(0) \frac{e^{-m_1 t} - e^{-m_0 t}}{m_1 - m_0}. \]

2. (Global existence) Now, we prove that $Q_2$ and $Q_3$ are bounded for $t \in I$. Using the bounds on $A_1$, $A_2$, $f_1$, $f_2$, the positivity of $Q_2$, $Q_3$ and the fact that $Q_1$ is bounded by an exponential function, we get 
\[ \frac{d}{dt} Q_2(t) \leq \tau_2 Q_2(t) \left( C - \frac{Q_0(t)}{\tau_2} \right) + M_1 e^{-\tau_2 t}, \]
where 
\[ C = 1 + \frac{m_2}{\tau_1} + \frac{m_2}{\tau_2}. \]
Moreover, the function $f : x \rightarrow ax(b - x)$ with $a > 0$ and $b \in \mathbb{R}$ is uniformly bounded from above for $x \in \mathbb{R}_+$, hence we obtain that $Q_2$ is bounded for $t \in I$. Similarly, using the bounds on $A_1$, $A_2$, $f_2$, the positivity of $Q_2$, $Q_3$ and the fact that $Q_2$ is bounded by a constant denoted $C_2(T)$ on $[0, T]$, we get 
\[ \frac{d}{dt} Q_3(t) \leq \tau_3 Q_3(t) \left( C - \frac{Q_0(t)}{\tau_3} \right) + M_2 C_2(T), \]
and that $Q_3$ is bounded for $t \in I$. Then the solution is global. 

**A.2. Limit system and asymptotic behavior** 

**A.2.1. Exponential convergence of the sensory axons** 

We first state an additional property of the solution. 

**Lemma 1 (Bounds on the Cancerous Cells).** 

Let $Q_0(0) > 0$, $Q_1(0) = Q_2(0) = Q_3(0) = 0$, $A_1(t) \in (-r_{A_1}, r_{A_1})$ and $A_2(t) \in (0, r_{A_2})$. 
\[ \forall t > r^* \quad c_s \leq Q_0(t) + Q_1(t) \leq C_s. \]

**Proof (Lemma 1).** We introduce the following notations: $y(t) = Q_2(t) + Q_3(t)$ and $C(A_1(t), A_2(t)) = 1 + \frac{A_1(t)}{\tau_3} + \frac{A_2(t)}{\tau_4}$. We recall that 
\[ \frac{d}{dt} y(t) = \tau_2 Q_2(t) + \tau_3 Q_3(t) \left( C(A_1(t), A_2(t)) - \frac{y(t)}{\tau_3} \right) + f_1(A_1(t)) Q_1(t). \]
Since $A_2(t) \leq r_{A_2}$ and $r_{A_1} < c_s$, there exists a constant $C_s$ such that 
\[ 0 \leq C(A_1(t), A_2(t)) < C_s. \]
Now, we assume there exists $t_0 > 0$ such that 
\[ y(t_0) > t_2 C_s + C \]
where $C_s > 0$ is a constant which will be discussed later and we denote $V_0$ a neighborhood of $y_0$. Using the bounds on $Q_0$ and $f_1$, we obtain 
\[ \frac{d}{dt} y(t) \leq \gamma y(t) \left( C_s - \frac{y(t)}{\tau_3} \right) + M_1 C(Q_0(t), Q_1(t)). \]
where $\gamma \in [\tau_2, \tau_3]$. The function $P : x \in \mathbb{R}_+ \mapsto \gamma x(C_s - x/\tau_3) + M_1 C(Q_0(t), Q_1(t))$ is polynomial which admits two roots: a negative and a positive one. We denote $y^+$ the positive root of $P$ and we assume $C$ large enough such that $y^+ < y(t_0)$. Then, for $t \in V_0$, we have 
\[ \frac{d}{dt} y(t) < 0 \quad \Rightarrow \quad y(t) \leq y(t_0). \]
Moreover, since the solutions of (8) are nonnegative, we obtain a uniform upper bound for $Q_2$ and $Q_3$. 

Now, we focus on the proof of the lower bound of $y$. First, we prove that it exists $t_1 \geq 0$ such that $y(t_1) > 0$. Let us assume that $\forall t \geq 0$, $y(t) = 0$. It implies that $\frac{d}{dt} y(t) = 0$ and that $Q_1$ is uniformly equal to 0. Moreover, $\frac{d}{dt} Q_1$ must be equal to 0 and then $\forall t \geq 0$ $Q_0(t) = 0$. It leads to a contradiction since $Q_0(0) > 0$.

Hence, let us assume that $0 < y(t_1) < c_0$ where $c_0 = 1 - \frac{\tau_{A_2}}{\tau_{c}}$ and then $c_0 \leq C(A_1(t), A_2(t))$. Moreover, we denote $V_1$ a neighborhood of $t_1$ and we have

$$\frac{d}{dt} y(t) = (\gamma_2 Q_3(t) + \gamma_3 Q_3(t)) \left( C(A_1(t), A_2(t)) - \frac{\gamma_1}{\tau_{c}} \right) + f_1(A_1(t))Q_1(t),$$

$$\geq \gamma_1 y(t) \left( c_0 - \frac{\gamma_0}{\tau_{c}} \right).$$

Then, for $t \in V_1$, we have

$$\frac{d}{dt} y(t) > 0 \implies y(t) \geq y(t_1).$$

**Proposition 3 (Exponential Convergence of the Sensory Axons).**

Let $Q_0(0) > 0$, $Q_3(0) = Q_2(0) = Q_1(0) = 0$, $A_1(0) \in (-\tau_{A_2}, \tau_{A_2})$ and $A_2(0) \in (0, \tau_{A_2})$. We assume $(H3)$ holds. Then $A_2$ tends exponentially fast to $\tau_{A_2}$, and for $r^* > 0$ large enough, there exist a constant $C > 0$ and a rate $r > 0$ such that

$$\forall t > r^*, \quad |A_2(t) - \tau_{A_2}| \leq C |A_2(0) - \tau_{A_2}| e^{-rt}.$$

**Proof.** Since the growth term of the sensory axons depends on the quantity of proliferating cells, the bounds on the cells populations $Q_2$ and $Q_3$ are one of the main information in order to deduce the exponential convergence of $A_2$. The following inequality gives us the bounds on the proliferating cells (PanIN and PDAC). Hence, Lemma 1 states that it exists $r^* > 0$ and two constants $0 < c_r < C_r$ such that

$$\forall t > r^*, \quad c_r \leq Q_2(t) + Q_3(t) \leq C_r.$$

We recall that

$$\frac{d}{dt} |A_2(t) - \tau_{A_2}| = \text{sign}(A_2(t) - \tau_{A_2})(\dot{\alpha_2} Q_2(t) + \dot{\alpha_3} Q_3(t)) A_2(t) \left( 1 - \frac{A_2(t)}{\tau_{A_2}} \right) = -\left(\dot{\alpha_2} Q_2(t) + \dot{\alpha_3} Q_3(t)\right) \frac{A_2(t)}{\tau_{A_2}} |A_2(t) - \tau_{A_2}|.$$
and then
\[ C(A^\infty_m) - \frac{f_m}{\tau_m} > 0. \]

Hence, we have
\[
0 = \gamma_3 Q_3^m \left( C(A^\infty_1) - \frac{Q_m^1 + Q_m^2}{\tau_2} \right) + f_m Q_2^m \frac{\gamma_3}{\tau_2} Q_3^m + f_m \frac{\gamma_3}{\tau_2} Q_3^m \left( C(A^\infty_1) - \frac{Q_m^1 + Q_m^2}{\tau_2} \right)
\]
and then
\[
Q_3^m = -\tau_3 \gamma_3 \left( C(A^\infty_1) - \frac{Q_m^1 + Q_m^2}{\tau_2} \right). 
\]

Since \( \gamma_2 < \gamma_3 \), we have that \( Q_3^m < 0 \) which positively

Finally, we obtain that \( Q_3^m = 0 \) and the steady states of \( Q_3^m = 0 \) and \( A^\infty_1 \) give the following Jacobian matrix:

\[
\begin{pmatrix}
\gamma_3(C(A^\infty_1) - \frac{Q_m^1 + Q_m^2}{\tau_2} - f_m) & 0 & 0 \\
-\gamma_3 \frac{Q_m^1 + Q_m^2}{\tau_2} + f_m & \gamma_3(C(A^\infty_1) - 2Q_m^2 / \tau_2) & \gamma_3 Q_m^2 / \tau_4 \\
\frac{\gamma_3}{\tau_1} - \gamma_3 & -\frac{\gamma_3}{\tau_1} + \frac{A^\infty_1}{\tau_1} & 2A^\infty_1 Q_m^2 / \tau_4
\end{pmatrix}
\]

In the case \( Q_3^m = Q_3^m \) and \( A^\infty_1 = -\tau_4 \), the eigenvalues of the Jacobian matrix are:

\[ \lambda_1 = -\gamma_3 C(A^\infty_1), \quad \lambda_2 = -\gamma_3 C(A^\infty_1), \quad \lambda_3 = 0. \]

In the last case, since \( Q_3^m = \tau_4 C(A^\infty_1) \) and \( A^\infty_1 = -\tau_4 \), the eigenvalues of the Jacobian matrix are:

\[ \lambda_1 = -f_m, \quad \lambda_2 = -\gamma_3 C(A^\infty_1), \quad \lambda_3 = 2\gamma_3 \tau_4 C(A^\infty_1) \frac{A^\infty_1}{\tau_4} \]

We conclude that the only linearly stable steady state is \( Q_3^m = 0 \), \( Q_3^m = \tau_4 C(-\tau_4), \quad A^\infty_1 = \tau_4 \). \( \square \)

Now, we study the global behavior of the limit system looking at the trajectories in the vector field. The system (12) is a modification of a competitive Lotka-Volterra system (Hofbauer et al., 1998) for \( Q_3^m \) and \( Q_3^m \) coupled to a modified logistic equation for \( A_1^\infty_1 \). The following result is established.

**Theorem 3** (Long Time Behavior of the Limit System (12)). Let (H2) and (H3) be true. Let \( Q_1(0) > 0 \) and \( A_1(0) \in \{-\tau_4, \tau_4\} \). Then \( X(t) = (Q_3(t), Q_3(t), A_1(t)) \rightarrow X^m = (0, \tau_4 C(-\tau_4), \tau_4) \) for \( t \rightarrow +\infty \)

where \( C(-\tau_4) = 1 - \frac{\gamma_3}{\tau_4} + \frac{\gamma_3}{\tau_4} \).

**Proof.** First, we introduce the following functions:

- \( z(t) = A_1(t), Q_2(t), Q_3(t) \) we also denote \( z(t) = z(A_1(t), Q_2(t), Q_3(t)) \) for the sake of simplicity,

- \( w(Q_2, Q_3) = a_2 Q_2 - a_1 Q_3 \), we also denote \( w(t) = w(Q_2(t), Q_3(t)) \) for the sake of simplicity.

Moreover, we introduce the following subdomains of \( \mathbb{R}_+ \times \mathbb{R}_+ \times \{-\tau_4, \tau_4\} \) (see Fig. 7):

- the domain 0a such that \( z = -f_2 / \tau_2 \geq 0, w \geq 0 \) and \( Q_1 \geq 0 \)
- the domain I such that \( z = -f_2 / \tau_2 \leq 0, w \geq 0, Q_1 \geq 0 \) and \( z \geq 0 \)
- the domain II such that \( z \leq 0, w \geq 0 \) and \( Q_1 \geq 0 \)
- the domain III such that \( z \leq -f_2 / \tau_2 \leq 0, w \leq 0, Q_1 \geq 0 \) and \( z \geq 0 \)
- the domain IV such that \( z \leq 0, w \leq 0 \) and \( Q_1 \geq 0 \)
- the domain 0b such that \( z = -f_2 / \tau_2 \leq 0, w \leq 0 \) and \( Q_1 \geq 0 \)

In Fig. 7, the red dots are the admissible steady states. In order to prove the convergence of \( X(t) \) toward \( X^m \), we look at the trajectories

in the different subdomains. We recall that it exists \( m, M \) such that \( 0 < m < f_2(t) < M \) for \( t \geq 0 \).

Now, let us suppose that it exists \( t_0 > 0 \) such that \( X(t_0) \in \theta_0^a \). Then \( 0 < m < f_2(t) < M \) for \( t \geq 0 \).

Let us suppose that it exists \( t_0 > 0 \) such that \( X(t_0) \in \theta_0^a \). Then \( 0 < m < f_2(t) < M \) for \( t \geq 0 \).

Then, the trajectories cannot stay in \( \theta_0^a \), since \( Q_1 \) is bounded (Lemma 1), and either the trajectories go to \( 0b \) or to \( I \). In addition, if the trajectories go from \( 0a \) to \( I \), it cannot go back again in \( 0a \). This result comes from the fact that \( \frac{d}{dt} Q_3 < 0 \) for \( X \in \theta_0 \cup I \) and the fact that if the vector field points toward \( I \) on \( z = 0, \frac{d}{dt} Q_3 = 0 \) when \( Q_1 = \tau \) where \( \tau > 0 \) then it points toward \( I \) on \( z = 0, \frac{d}{dt} Q_3 = 0 \) and \( \{ Q_3 \geq \tau \} \) (Lemma 2).

Let us suppose that it exists \( t_1 > 0 \) such that \( X(t_1) \in I^b \). Then \( 3\delta > 0 \) such that for \( t \in [t_1, t_2 + \delta] \),

\[ \frac{d}{dt} Q_3(t) < 0, \quad \frac{d}{dt} A_1(t) < 0. \]

The trajectories cannot stay in \( 0b \) and go to \( III \) since the null steady state is locally unstable (Proposition 4) and \( Q_2 \) and \( Q_3 \) are non-decreasing. Moreover, the vector field points toward \( III \) on \( z = -f_2 / \tau_2 = 0 \).

If \( X(t_1) \in III^b \) then \( 3\delta > 0 \) such that for \( t \in [t_2, t_2 + \delta] \),

\[ \frac{d}{dt} Q_2(t) < 0, \quad \frac{d}{dt} A_1(t) < 0. \]

The trajectories cannot stay in \( 0b \) and go to \( III \) since the null steady state is locally unstable (Proposition 4) and \( Q_2 \) and \( Q_3 \) are non-decreasing. Moreover, the vector field points toward \( III \) on \( z = -f_2 / \tau_2 = 0 \).

In addition, if there exists \( t_4 > 0 \) such that \( X(t_4) \in III^b \) then \( 3\delta > 0 \) such that for \( t \in [t_4, t_4 + \delta] \),

\[ \frac{d}{dt} Q_3(t) < 0, \quad \frac{d}{dt} A_1(t) < 0. \]

Let us suppose that the trajectories remains in \( II \). Since \( Q_2, Q_3 \) and \( A_1 \) are non-monotonous and bounded, \( X \) converge to a point in \( II \). However, it is absurd since there is no steady state in \( II \). It implies that the trajectories leave the space \( II \) and enter either I, III or IV. \( \square \)
Lemma 2 (Study of the Vector Field of (12)). Let \( z = 0, \omega = 0 \) and \( \frac{\partial \pi}{\partial \pi \frac{G(y)}{v}} = 0 \) be the three surfaces of interest in order to study the vector field of the system (12).

\[
\begin{align*}
\pi \cdot f(Q_2, Q_3, A) &= a_2 Q_2 \left( y_2 - y_3 \right) - \left( 1 + \frac{a_2}{a_2} \right) f_2(A_1), \\
\pi \cdot f(Q_2, Q_1, A) &= \frac{1}{\tau_A} \left( 1 - \frac{A_1}{\tau_A} \right)^2, \\
\pi \cdot f(Q_2, Q_1) &= -\frac{1}{\tau_C} f_2(A_1) \left( \frac{A_1}{\tau_A} + Q_2 \right) + \left( \frac{1}{\tau_C} f(A_1) \right) \left( 1 - \frac{A_1}{\tau_A} \right)^2.
\end{align*}
\]

\( \omega \)

\[
\begin{align*}
\pi \cdot f(Q_2, Q_3, A_1) &= \frac{1}{\tau_A} \left( 1 - \frac{A_1}{\tau_A} \right)^2, \\
\pi \cdot f(Q_2, Q_1, A_1) &= -\frac{1}{\tau_C} f_2(A_1) \left( \frac{A_1}{\tau_A} + Q_2 \right) + \left( \frac{1}{\tau_C} f(A_1) \right) \left( 1 - \frac{A_1}{\tau_A} \right)^2.
\end{align*}
\]

A.2.3. Asymptotic behavior of the complete system

Once the convergence is established on the limit system, the global asymptotic behavior of the complete system is given by the following theorem.

Theorem 4 (Long Time Behavior of the System (8)).

Let \( Q_0(0) > 0, Q_0(0) \geq 0 \) for \( t = 1, 2, 3 \) and \( A_1(0) \in (-\tau_A, \tau_A) \) and \( A_2(0) \in (0, \tau_A) \). Let (H2) and (H3) be true. Then

\[
X(t) = \left( Q_0(t), Q_1(t), Q_2(t), Q_3(t), A_1(t), A_2(t) \right) \\
\longrightarrow \left( 0, 0, 0, \tau_C C(-\tau_A), -\tau_A, \tau_A \right)
\quad \text{for } t \rightarrow +\infty
\]

where \( C(-\tau_A) = 1 - \frac{\tau_A}{\tau_A} + \frac{\tau_A}{\tau_A} \).

Proof. We introduce the function \( F : (0, \infty) \times \mathbb{R}^3 \rightarrow \mathbb{R} \) such that for \( Y = \left( Q_2, Q_3, A_1 \right) \):

\[
F(t, Y) = g_1 Q_1 \left( 1 - \frac{Q_1 + Q_2}{\tau_A} + \frac{A_1}{\tau_A} + \frac{A_2}{\tau_A} \right) f_1 \left( A_1, t \right) Q_1(t) - f_2 \left( A_1, A_2(t) \right) Q_2 + \\
\left( A_1 Q_1(t) + a_2 Q_2 - a_2 Q_1 \right) \left( 1 + \frac{a_2}{\tau_A} \right) \left( 1 - \frac{A_1}{\tau_A} \right)
\]

Moreover, Proposition 1 gives the exponential convergence of \( Q_0 \) and \( Q_1 \) toward 0 and Proposition 3 gives the exponential convergence of \( A_1 \) toward \( \tau_A \). Hence, we obtain that

\[
F(t, Y) \rightarrow G(Y) \quad \text{as } t \rightarrow \infty \quad \text{uniformly locally in } Y \in \mathbb{R}^3,
\]

where

\[
G(Y) = g_1 Q_1 \left( 1 - \frac{Q_1 + Q_2}{\tau_A} + \frac{A_1}{\tau_A} + \frac{A_2}{\tau_A} \right) f_1 \left( A_1, \tau_A \right) Q_2 + \\
\left( a_2 Q_2 - a_2 Q_1 \right) \left( 1 + \frac{a_2}{\tau_A} \right) \left( 1 - \frac{A_1}{\tau_A} \right)
\]

Using the results of Theorem 3, we have that the \( \omega \)-limit set (see Appendix B, Eq. (21)) of the limit system (12) is restricted to

\[
\omega(0, y_0) = \{ (0, 0, C(-\tau_A), -\tau_A, \tau_A) \}.
\]

Then, using Theorem 6 on the asymptotically autonomous differential equations (see Appendix B), we deduce the following results on the solutions of system (8)

\[
(0, 0, 0, \tau_C C(-\tau_A), -\tau_A, \tau_A) \quad \text{for } t \rightarrow +\infty.
\]

Appendix B. Results on asymptotically autonomous differential systems

In this section, we recall some results on asymptotically autonomous differential equations. The proofs of the results and further details can be found in Markus (2016), Thieme (1994, 1992).

Definition 1. Let \( f : \mathbb{R} \times \mathbb{R}^n \rightarrow \mathbb{R}^n \) and \( g : \mathbb{R}^n \rightarrow \mathbb{R}^n \) be continuous and locally Lipschitz on \( \mathbb{R}^n \). An ordinary differential equation in \( \mathbb{R}^n \nabla x = f(t, x), \)

is called asymptotically autonomous with limit equation

\[
\dot{y} = g(y),
\]

if

\[
f(t, x) \xrightarrow{t \rightarrow \infty} g(x), \quad \text{locally uniformly in } x \in \mathbb{R}^n.
\]

We denote the \( \omega \)-limit set of \( \omega \) of a forward bounded solution \( x \) to (19) satisfying \( x(t_0) = x_0 \) by \( \omega(x_0) \):

\[
\omega(x_0) = \bigcup \{ x(t) : t \geq 0 \}.
\]

We recall the main theorems established by Markus in Markus (2016).

Theorem 5. The \( \omega \)-limit set of \( \omega \) of a forward bounded solution \( x \) to (19) is nonempty, compact, and connected. Moreover

\[
dist(x(t), \omega) \xrightarrow{t \rightarrow \infty} 0.
\]

Finally \( \omega \) is invariant under (20), i.e., \( \dot{y}(t) = y_0 \) is nonempty, compact, and connected. Moreover

These theorems have been used in population dynamics in order to prove that asymptotically autonomous ODEs arising from the models converge to equilibrium (e.g. Castillo-Chavez and Thieme 1994). Moreover, these theorems have been generalized in Thieme (1992) to be applied for specific PDEs.

Appendix C. Methods for the identifiability analysis with the profile likelihood

C.1. Parameters of the model

Table 2 describes the list of all parameters appearing in the model equations. Each parameter is supplied with its range of values and a short description. The range of parameter values is chosen to be large for two reasons. The first is not to impose too restrictive conditions since the model is completely original and very little information is
Numerical computation of the profile likelihood for nine parameters. Each row corresponds to a parameter. This parameter takes twenty distinct values represented by the color gradient. The numerical method gives a cost distribution for each value of the parameter. This distribution is then visualized by a boxplot (the vertical axis represents the cost). This method is iterative for the non-identifiable parameters and this leads to a new simulation on a reduced optimization domain. For instance, $\pi_0$, $\pi_1$, and $\gamma_2$ are identifiable from the first iteration. The parameters $\pi_2$ and $\delta_0$ are identifiable from the second iteration and $\gamma_3$ from the third iteration. The other parameters are non-identifiable after three iteration.

Numerical computation of the profile likelihood for the other parameters. Each row corresponds to a parameter. This parameter takes twenty distinct values represented by the color gradient. The numerical method gives a cost distribution for each value of the parameter. This distribution is then visualized by a boxplot (the vertical axis represents the cost). This method is iterative for the non-identifiable parameters and this leads to a new simulation on a reduced optimization domain. For instance, only $\tau_C$ is identifiable (from the second iteration). The other parameters are non-identifiable.

Available. The second is to maximize the search space for reasonable parameters in order to calibrate the model. It is then possible to divide the parameters of the model to estimate into five categories:

1. **The transfer rates** $\pi_0$, $\pi_1$, and $\pi_2$. These parameters describe the main rates of cell transfer. More specifically, the time evolution of cell and axon populations is simulated by a compartmental model and these parameters quantify the speed of transfer from one to another compartment.

2. **The proliferation speeds** $\gamma_2$ and $\gamma_3$. These parameters give the growth speeds of the PanIN and PDAC cell populations.

3. **The parameters of the regulations on the transfer rates** $\beta_1$, $\beta_2$, $\delta_0$ and $\delta_2$. These parameters are multiplicative coefficients
appearing in the transfer rates. They regulate either positively or negatively the speed of transfer from one compartment to another in the model.

4. The saturation rates $τ_{c1}$, $τ_{c2}$, $τ_{a1}$ and $τ_{a2}$. The saturation rates are closely linked to bio-physical constraints such as the maximal volume of the model’s domain (i.e. the pancreas) and the maximal axon densities in the domain. These two quantities might vary from one individual to another, however one can reasonably assume maximal bounds and implement it in the model.

5. The parameters of the regulations on the proliferation speeds of axons $a_1$, $a_2$, $a_3$, $a_4$ and $a_5$. These parameters are multiplicative coefficients appearing in the growth terms of the axons. Their main effect is to modulate the rate of axon proliferation depending on the amount of cell populations present at the observed time.

Moreover, the density of autonomic axons in a healthy pancreas $A_1^{\text{th}}$ is given by the control experiments and amounts to $0.980 \text{ (nm (μm)}^{-3}\text{)}$. The other parameter set in this model is $τ_{a1}$ (i.e. the saturation rate of $A_1$). Since $A_1$ is the variation of the density of autonomic axons with respect to $A_1^{\text{th}}$, setting the value of $τ_{a1}$ to 0.3 implies that the maximum variation of the density of autonomic axons does not exceed one third of its equilibrium state. This assumption can be justified in the model by the fact that natural innervation and denervation in vivo appear to be phenomena at the margin during the development of the pancreatic adenocarcinoma. In addition, autonomic axons appear to be pushed to the periphery of the organ during the development of PDAC cells and the domain of the model is the pancreas and its immediate surroundings.

C.2. Numerical method to assess the identifiability with the profile likelihood

In this section, we detail the method used to study the identifiability of the parameters. It is assimilated to an optimization problem and can be considered as a one-at-a-time identifiability process since we study the profile likelihood of each parameter independently. We recall that the parameters are denoted by the vector $θ$ which include all the parameters except for $τ_{a4}$ and $A_1^{\text{th}}$ fixed as in Table 2. The computation of the profile likelihood is broken down as follows:

**Step 1.** For each component $θ_j$ of the vector of parameters, we choose a sequence of 20 values which discretizes its range of values. The sequence discretize uniformly the range of values of the parameters $δ_{j_1}, β_1, β_2, τ_{c1}, τ_{c2}, \bar{τ}_{a1}$ and $\bar{τ}_{a2}$. For the other parameters, the sequence discretize uniformly the $\log_{10}$ transformation of their respective ranges of value. This allows us to explore more precisely the optimization domain by taking into account the differences in the orders of magnitude between parameters. The sequence of values chosen for the parameter $θ_j$ is denoted $(p_j^i)_{i=1\ldots20}$.

**Step 2.** For each $p_j^i$, we minimize $Χ^2_j$ defined by (16) on $θ ∈ \left\{θ \mid θ_j = p_j^i \right\}$. Since, the problem is non-linear, non-convex and high dimensional, we choose to repeat 50 times the optimization procedure using as the initialization step a random set of parameters chosen by the uniform distribution over their respective value range (or over the $\log_{10}$ transformation of their value range). For this optimization problem, we use the CMA evolution strategy algorithm (Hansen, 2016) : at each step of the optimization loop, the algorithm picks a set of parameters given by a specific random distribution over the optimization domain. It evaluates the objective function value for this set of parameters and iterate by updating the random distribution with the barycenter of the “best” parameters (i.e. whose objective function

<table>
<thead>
<tr>
<th>Description</th>
<th>Symbol</th>
<th>Units</th>
<th>Range of value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average density of autonomic axons in healthy pancreas</td>
<td>$A_1^{\text{th}}$</td>
<td>nm (μm$^{-3}$)</td>
<td>0.0999</td>
</tr>
<tr>
<td>Saturation term of the logistic-like growth</td>
<td>$τ_{c1}$</td>
<td>nm (μm$^{-3}$)</td>
<td>0.3</td>
</tr>
<tr>
<td>Transfer rate of the $Q_0$ to $Q_1$</td>
<td>$a_0$</td>
<td>day$^{-1}$</td>
<td>$(10^{-3}, 1)$</td>
</tr>
<tr>
<td>Transfer rate of the $Q_1$ to $Q_2$</td>
<td>$a_1$</td>
<td>day$^{-1}$</td>
<td>$(10^{-3}, 1)$</td>
</tr>
<tr>
<td>Transfer rate of the $Q_2$ to $Q_3$</td>
<td>$a_2$</td>
<td>day$^{-1}$</td>
<td>$(10^{-3}, 1)$</td>
</tr>
<tr>
<td>Growth rate of $Q_2$</td>
<td>$τ_2$</td>
<td>day$^{-1}$</td>
<td>$(10^{-4}, 1)$</td>
</tr>
<tr>
<td>Growth rate of $Q_3$</td>
<td>$τ_3$</td>
<td>day$^{-1}$</td>
<td>$(10^{-4}, 1)$</td>
</tr>
<tr>
<td>Amplitude of the effect of $A_1$ on the transfer rate</td>
<td>$β_1$</td>
<td>nm$^{-1}$ μm$^{-1}$</td>
<td>$(0, 10^{-1})^2$</td>
</tr>
<tr>
<td>Amplitude of the effect of $A_2$ on the transfer rate</td>
<td>$β_2$</td>
<td>nm$^{-1}$ μm$^{-1}$</td>
<td>$(0, 10^{-1})^2$</td>
</tr>
<tr>
<td>Maximum amplitude of the Michaelis-Menten term</td>
<td>$a_0$</td>
<td>unitless</td>
<td>$(0.10)$</td>
</tr>
<tr>
<td>Amplitude of the effect of $A_1$ on the transfer rate</td>
<td>$α_1$</td>
<td>nm$^{-1}$ μm$^{-1}$</td>
<td>$(0.10)$</td>
</tr>
<tr>
<td>Saturation term of the logistic growth</td>
<td>$τ_{c1}$</td>
<td>cells (nm$^{-3}$)</td>
<td>$(50, 10^3)$</td>
</tr>
<tr>
<td>Saturation term of the logistic growth</td>
<td>$τ_{c2}$</td>
<td>nm (μm$^{-3}$)</td>
<td>$(0, 2.0)$</td>
</tr>
<tr>
<td>Threshold for the effect of $A_1$ on the growth of $Q_2$</td>
<td>$τ_{a1}$</td>
<td>nm (μm$^{-3}$)</td>
<td>$(0.3, 2)$</td>
</tr>
<tr>
<td>Threshold for the effect of $A_1$ on the growth of $Q_2$ and $Q_3$</td>
<td>$τ_{a2}$</td>
<td>nm (μm$^{-3}$)</td>
<td>$(0, 0.2)$</td>
</tr>
<tr>
<td>Amplitude of the effect of $Q_0$ on the growth of $A_1$</td>
<td>$a_1$</td>
<td>mm$^{-1}$ cell$^{-1}$ day$^{-1}$ nm (μm$^{-1}$)</td>
<td>$(10^{-3}, 1)$</td>
</tr>
<tr>
<td>Amplitude of the effect of $Q_1$ on the growth of $A_1$</td>
<td>$a_2$</td>
<td>mm$^{-1}$ cell$^{-1}$ day$^{-1}$ nm (μm$^{-1}$)</td>
<td>$(10^{-3}, 1)$</td>
</tr>
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<td>Amplitude of the effect of $Q_0$ on the growth of $A_1$</td>
<td>$a_3$</td>
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<td>$(10^{-3}, 1)$</td>
</tr>
<tr>
<td>Amplitude of the effect of $Q_0$ on the growth of $A_1$</td>
<td>$a_4$</td>
<td>mm$^{-1}$ cell$^{-1}$ day$^{-1}$</td>
<td>$(10^{-3}, 1)$</td>
</tr>
</tbody>
</table>

Table 3

Table of parameters for the numerical computations in Fig. 3.
values are the lowest). The algorithm stops if the distance between the best evaluation of the objective function of the last 40 iterations of the optimization loop and all the objective function values of the last iteration is less than the tolerance threshold $10^{-3}$.

Step 3. For each $\theta_j$, we plot the first quartile, the last quartile and the median of the 50 optimal objective function values obtained in order to visualize the results of step 2. It gives the following Figs. 8–9. If the curves formed by the median describe a convex shape and a minimum is clearly attained in a distinct value, we consider that the parameter is identifiable and its estimate is this distinct values (for instance, in Fig. 8, the top panel shows that median attains its minimal value at $\theta_j \approx 0.0025$). Else, we iterate step 2 once or twice on a reduced domain where the already identifiable parameters are fixed to their estimated values.

The numerical results of this optimization process are summarized in Figs. 8–9. We denote trial 1 the first iteration of step 2, trial 2 the second and trial 3 the third. In total, seven parameters are identifiable (see Fig. 4 and Table 1). We choose to stop iterating the identifiability process after the third trial considering the amount of data at our disposal and the over fitting issues coming from the high dimensionality of our problem. One can reasonably justify this choice by looking at the distribution of the objective function values in trial 3 (see Figs. 8–9). The cost function values over the whole range for the remaining parameters are concentrated under 10 and the median of the cost distribution does not have a clearly distinguishable minimum value. An extra iteration might not ensure a sufficient difference between the minimum of the median and the rest of the median values.

As a final validation step, we perform the following numerical experiments: we fix the seven identifiable parameters to their values (see Table 1), we pick 200 sets of rescaled parameters (for the non identifiable ones) from a uniform random distribution over their value ranges and we compute the minimization problem

$$C = \min_{\theta \in \mathbb{R}^{|\mathcal{P}|-|\mathcal{I}|}} J(\theta)$$

where $J_\theta$ is defined by (16) and $\mathcal{I}$ is the set of coordinates of the identifiable parameters denoted $\mathcal{P} \setminus \mathcal{I}$. It follows that the distribution of the optimal objective function evaluations is concentrated on 3 (see Fig. 10). For instance, the costs of the trajectories shown in Fig. 3 are superior or equal to 3. This numerical experiments ensures that the results on the identifiable parameters are satisfactory from a qualitative point of view.

References


