A control engineering approach to understanding the TGF-β paradox in cancer

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TGF-β, a key cytokine that regulates diverse cellular processes, including proliferation and apoptosis, appears to function paradoxically as a tumour suppressor in normal cells, and as a tumour promoter in cancer cells, but the mechanisms underlying such contradictory roles remain unknown. In particular, given that this cytokine is primarily a tumour suppressor, the conundrum of the unusually high level of TGF-β observed in the primary cancer tissue and blood samples of cancer patients with the worst prognosis, remains unresolved. To provide a quantitative explanation of these paradoxical observations, we present, from a control theory perspective, a mechanistic model of TGF-β-driven regulation of cell homeostasis. Analysis of the overall system model yields quantitative insight into how cell population is regulated, enabling us to propose a plausible explanation for the paradox: with the tumour suppressor role of TGF-β unchanged from normal to cancer cells, we demonstrate that the observed increased level of TGF-β is an effect of cancer cell phenotypic progression (specifically, acquired TGF-β resistance), not the cause. We are thus able to explain precisely why the clinically observed correlation between elevated TGF-β levels and poor prognosis is in fact consistent with TGF-β’s original (and unchanged) role as a tumour suppressor.

Keywords: TGF-β; cancer; control theory; tissue homeostasis

1. INTRODUCTION

Normal tissue homeostasis is maintained by a delicate and dynamic balance between the cellular processes of proliferation and death. In particular, too much growth and too little death can lead to a severe condition that may ultimately result in cancer [1]. These cellular processes are affected by a variety of extracellular stimuli, each capable of inducing its own set of responses via specific intracellular signalling cascades. Among these extracellular signals, transforming growth factor β (TGF-β) has drawn much attention from cancer researchers because it plays a central role in regulating both cell proliferation and cell death in the tumour cell microenvironment [2–4].

TGF-β is an important participant in a variety of physiological processes in both normal and malignant tissues [3], but considerable debate remains over its exact role during cancer progression. During the early stages of epithelial tumorigenesis, that TGF-β functions as a potent tumour suppressor primarily by inducing cell cycle arrest and programmed cell death (i.e. apoptosis) is unquestioned. However, the level of TGF-β is frequently elevated in many malignant tissues and in blood samples from cancer patients with the worst prognosis. As such, the role of TGF-β in the late phases of tumour progression appears to become—somehow—one of tumour promotion, by appearing to support cell proliferation, by subverting immune surveillance and also facilitating epithelial to mesenchymal transition, invasion and angiogenesis [5]. This has created the widely held perception that TGF-β is simultaneously a tumour suppressor under one condition and a tumour promoter under another. If this is true, how does a single stimulus produce multiple contradictory results? A clearer understanding of these apparently contradictory roles of TGF-β in cancer requires quantitative results, because TGF-β biology is far too complex to be understood based on qualitative descriptions alone.

While extensive physiological, biochemical and clinical information is available on TGF-β, quantitative modelling of the TGF-β signalling system remains comparatively in its infancy. Furthermore, as briefly reviewed in Chung et al. [6], all published computational

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TGF-β models to date have focused on the intracellular signal transduction pathway via Smad transcription factors in a single cell. Such single-cell models, despite their significant contributions to our understanding of the dynamic behaviour of TGF-β signalling, are not sufficient to interpret the contradictory clinical observations seen in cancer patients. The dynamic characteristics of extracellular molecules and signals depend strongly on the active interactions among cells and/or between cells and the surroundings. As such, providing satisfactory explanations for these extracellular dynamics requires more than individual studies of intracellular events in isolation. Thus, a more realistic understanding of the role of TGF-β in cancer requires a more comprehensive examination of the TGF-β system encompassing the cells in the context of their microenvironment.

To this end, we present in this study a macroscopic mechanistic model of TGF-β-driven regulation of cell homeostasis. The model deals, not with a single cell, but with the cell population as a systemic entity, and represents a control system characterization of how TGF-β achieves cell homeostasis via communication between the cell population and its microenvironment. We begin by identifying the various functional components of the system, their respective input and output variables, and how they connect to form the complete control system; each component then is modelled on the basis of available consensus information in the reported biological literature. Where the required information is unavailable, we provide and employ reasonable assumptions to support our postulates adequately. The resulting overall system model then is analysed to obtain quantitative insight into how the biological processes of cell proliferation and death are regulated by TGF-β. The model also allows us to predict possible dynamic characteristics of the TGF-β-mediated control system in cancer tissues, from which we present an alternative perspective of the TGF-β paradox in cancer. Finally, we note that alternative expressions different from the ones we have chosen may be similarly valid for describing various components of the system; however, so long as these alternative expressions adequately reflect—and are consistent with—physiological reality, there is no reason to expect any qualitative difference in the final results.

2. MODEL DEVELOPMENT

Of all the physiological processes that influence homeostasis in a cell population, none is as critical as the combined processes of cell proliferation and death. Maintaining the dynamic balance between proliferation and death regulates cell population dynamics; and biological regulation is achieved in general by dedicated biological control systems. In the specific case of this study, we restrict our attention to the TGF-β-mediated system for achieving cell homeostasis, viewed as an automatic biological control system for rejecting ‘disturbances’ that will otherwise provoke a cell population to grow indefinitely and become cancerous if invasiveness is subsequently acquired as an inherent trait [1]. As with all control systems, engineered or biological, this control system will also consist of at least the following component subsystems: (i) sensor: receives information about the ‘controlled process’ state and generates appropriate signals that are transmitted to the regulatory machinery; (ii) controller: the regulatory machinery that receives ‘process state’ signals and generates appropriate corrective action signals; and (iii) actuator: the ‘final control element’ that implements the corrective action on the controlled process. By representing each functional component with a block showing inputs and outputs as determined from mechanistic information available in the literature (and discussed in detail subsequently), the overall control system block diagram is shown in figure 1. What follows is a detailed discussion of model development for each component subsystem. While we recognize that cell populations in general are heterogeneous, and, for that matter, that cancer cells derived from a single tumour are frequently genetically inhomogeneous, the following model does not deal with heterogeneity explicitly; rather, the cell
population is characterized by ensemble average characteristics so that our models occur in the form of deterministic ordinary differential equations. A future follow-up study will address cell population heterogeneity explicitly using a stochastic modelling approach and will explore what effects, if any, cell population heterogeneity has on the conclusions reached in this current study.

2.1. Controlled process: cell proliferation and death

In this study, the controlled process is the combined biological process of cell proliferation and death. The output of interest—the ‘controlled output’—is the total cell population count. Our study is restricted to cell proliferation and death as regulated by TGF-β via its ability to inhibit cell proliferation and induce apoptosis. As such, the ‘manipulated input’ is the amount of bioactive TGF-β to which the cell population is exposed. Because the specific pathology of interest is cancer, pro-proliferative signals (such as growth factors and hormones) constitute the ‘disturbance’ of interest whose effects on proliferation are to be handled appropriately by the TGF-β-mediated control system, if normal cell growth and proliferation is to be kept under judicious restraint. The desired mathematical model therefore will represent the response of cell population to stimulation by growth factors, on one hand, and bioavailable TGF-β, on the other hand.

Cell population dynamics are modelled under the following simplifying assumptions: (i) all cells of interest are capable of proliferating and do so at a uniform rate, \( p \); (ii) all cells are readily and equally accessible to extracellular stimuli; (iii) cell death occurs at a uniform rate, \( d \), for all cells; (iv) cell population dynamics are dominated by proliferation and death so that other cellular processes, including differentiation and migration, can be neglected; and (v) upon initial stimulation with growth signals, cells start to proliferate immediately, with no delay, and enter successive and synchronous cell division rounds thereafter.

The simplest model consistent with these assumptions is

\[
\frac{dX}{dt} = (p - d)X,
\]

where \( X \) is the total number of cells in the population. Observe that when \( p = d \), the cell population is at steady state; when \( p > d \), the population either grows or shrinks exponentially, depending on whether \( p > d \) or vice versa. The population dynamics are therefore clearly determined by the parameters \( p \) and \( d \), which, in turn, are determined by the level of the extracellular cues that induce proliferation or death.

First, the rate of cell proliferation, \( p \), is known to increase with the level of proliferation stimuli, but decreases with the level of anti-proliferation factors such as TGF-β which inhibits clonal expansion by arresting the cell cycle in the G1 phase [3,7]. Therefore, we postulate the following functional relationship between the rate of cell proliferation, \( p \), and the concentrations of growth factors and TGF-β as two distinct terms, assuming no interaction effect between these distinct stimuli:

\[
p(GF, TGFβ) = \frac{p_b \cdot GF^r}{p_b^l + GF^r} - \frac{p_n^v \cdot TGFβ^m}{p_n^v + TGFβ^m}, \quad p \geq 0.
\]

(2.2)

Here, \( GF \) and \( TGFβ \) denote the concentrations of growth stimuli of any kind and of TGF-β, respectively; \( p_b \) is the maximum cell division rate; \( p_n \) is the maximum anti-growth rate, where \( r \) represents the effectiveness of the TGF-β-induced cytostasis; \( p_b \) and \( p_n \) are affinity constants; \( r \) and \( m \) are Hill coefficients.

Next, \( d \), the rate of cell death, is influenced by the level of pro-apoptotic stimuli such as the TNF superfamily; it is also known that TGF-β promotes the death of unhealthy, damaged and unnecessary cells by inducing apoptosis [3]. Thus, the rate of cell death should increase with higher TGF-β levels. Consequently, we represent the dependence of \( d \) on TGF-β level as follows:

\[
d(TGFβ) = d_1 + \frac{d_2 \cdot TGFβ^n}{d_3 + TGFβ^n},
\]

(2.3)

where \( d_1 \) is the inherent rate of death due to endogenous pro-apoptotic factors; \( d_2 \) represents the maximum rate of TGF-β-induced apoptosis, and \( n \) denotes the effectiveness of the TGF-β-induced apoptosis; \( d_3 \) is an affinity constant; and \( n \) is a Hill coefficient.

The overall model equation for the controlled process is therefore:

\[
\frac{dX}{dt} = (p - d)X - \left( \frac{p_b \cdot GF^r}{p_b^l + GF^r} - \frac{p_n^v \cdot TGFβ^m}{p_n^v + TGFβ^m} - d_1 - \frac{d_2 \cdot TGFβ^n}{d_3 + TGFβ^n} \right) \cdot X.
\]

(2.4)

2.2. Sensor/controller: TGF-β production system

To elicit the well-established physiological response of healthy tissue to unusual changes in its cell population size, such changes must be detected by some sort of ‘sensor’ system, which in turn will stimulate the required response from the ‘biological controller’ responsible for maintaining cellular homeostasis. In this particular case, the response of interest is the production of TGF-β for the express purpose of restraining unusual growth. While the precise mechanisms by which this purpose is achieved remain incomplete, a growing body of knowledge is emerging to provide clues concerning the basic characteristics.

When cells undergoing unusual growth break through the basement membrane, (i) they encounter the stroma, resulting in local inflammation; (ii) in response, TGF-β is produced locally in a latent form—known as large latent complexes (LLCs), which consists of latency-associated propeptide (LAP), active TGF-β and a latent TGF-β-binding protein (LTBP) [8–10]—in the stroma, including various immune cell types, such as macrophages, dendritic cells, T cells, B cells, etc. [11]; and finally, (iii) bioactive TGF-β is made available by a
subsequent multi-step process of activation (including secretion, interaction with extracellular matrix (ECM) components and proteolytic cleavage), with each step in the activation process under tight control [10]. Under normal circumstances, this action is sufficient to eliminate the errant cells, repair the damage and promote normal healing.

Thus, even though not all of the mechanistic details of how the TGF-β producer cells ‘monitor and respond’ to unusual changes in cell population are known, it is clear from what is known that such a TGF-β production system is the biological controller; and that it appears to be stimulated directly by changes in cell population. As such, we consider the total cell number, \( X \), as the input to this combined sensor/controller, and the output is the level of inactive TGF-β complex produced in response to changes in the total cell number. (Modelling how active TGF-β is produced from the latent form is discussed later.)

As an anti-growth cytokine, the level of TGF-β should increase with growing cell population in order to inhibit abnormal cell growth. Conversely, a decrease in cell number should result in a commensurate reduction in the level of TGF-β. Therefore, as presented in many physiology textbooks [12], we employ the following sigmoidal response function for this biological controller:

\[
\text{LLC}(X) = \frac{K}{1 + e^{C_3(C_4 - X)}} \tag{2.5}
\]

indicating how LLC, the inactive TGF-β complex concentration, changes as a function of \( X \), the cell population. Here, \( K \) is the maximum level of latent TGF-β, \( C_a \) is a scaling parameter, and \( C_3 \) is the sigmoid’s ‘centre parameter’ at which the controller output is half of the maximum value, \( K \). It can be shown that for this nonlinear controller, the effective controller gain, \( \partial(\text{LLC})/\partial X \), is maximum at \( X = C_3 \); also, it can be shown that \( C_3 \) is the ‘implicit set-point’ around which the controller wants to maintain \( X \).

### 2.3. Actuator: TGF-β activation system

As noted briefly above, in response to changes in cell population, TGF-β producer cells secrete an inactive form of the cytokine that is easily bound to and stored in ECM proteins (e.g. fibrillin-1, perlecan/HSPG2 and fibronectin) via its latent LTBP component. In order to become bioactive (i.e. to be able to bind to its cognate cell-surface receptors and trigger intracellular signalling), TGF-β proteins sequestered within the ECM-bound LLC need to be released [10]. The dissociation of bioactive TGF-β from the LLC–ECM complex is mediated by two distinct mechanisms: enzymatic (or proteolytic) and mechanical. Enzymatic cleavage involves a variety of proteases, including metalloproteinases and serine proteases (e.g. plasmin, thrombin, tryptases, etc.), and appears to be the most prominent of the two mechanisms [13]. With mechanical dissociation, TGF-β is released by cell traction forces generated via integrins that bind to an LLC component known as LAP [14].

Our model of this TGF-β activation process is based on these two mechanisms, along with the following considerations: (i) TGF-β bioavailability depends most strongly on the final step of the activation process, the release of TGF-β from its latent complex; (ii) this protease-driven dissociation follows Michaelis–Menten kinetics; (iii) integrins that mediate cell traction force exhibit enzyme-like activity with Michaelis–Menten kinetics; and (iv) the released bioactive TGF-β can be irreversibly degraded by endogenous proteases.

This leads to the following ‘actuation dynamics’ equation, representing the dynamics of activated TGF-β as a function of the concentration of the controller output, LLC concentration, and the levels of participating proteases and integrins.

\[
\frac{d\text{TGFβ}}{dt} = \frac{k_{\text{cat1}} \cdot P \cdot \text{LLC}}{K_{\text{n1}} + \text{LLC}} + \frac{k_{\text{cat2}} \cdot I \cdot \text{LLC}}{K_{\text{n2}} + \text{LLC}} - k_{\text{deg}} \cdot \text{TGFβ}. \tag{2.6}
\]

Here, \( P \) and \( I \) denote the concentrations of TGF-β-activating proteases and integrins, respectively; \( k_{\text{cat1}} \) and \( k_{\text{cat2}} \) are turnover numbers; \( K_{\text{n1}} \) and \( K_{\text{n2}} \) are Michaelis–Menten constants; and \( k_{\text{deg}} \) is the rate of proteolytic degradation of bioactive TGF-β.

Finally, as part of the cell population regulation process, proliferating cells themselves produce proteases that promote the activation of TGF-β. In addition, proliferating cells also provide cell-surface integrins that potentially can associate directly with, and hence promote the activation of, latent TGF-β. The activation process therefore involves feedback loops from the controlled process itself through the proteases and integrins from proliferating cells (figure 1). Consequently, in the absence of further mechanistic information, the simplest way to represent the concentrations of active proteases, \( P(t) \), and of integrins, \( I(t) \), is with the following equations:

\[
P(t) = k_p \cdot X(t) + \text{PRT}_0 \tag{2.7}
\]

and

\[
I(t) = k_i \cdot X(t), \tag{2.8}
\]

where \( X \) is the total cell number (as in equation (2.1)); \( k_p \) is a proportional constant for protease synthesis/activity from proliferating cells; \( \text{PRT}_0 \) is the constitutive production level of proteases in the tissue; and \( k_i \) is a proportional constant for the number of integrins from a cell that can potentially bind to latent TGF-β.

### 2.4. Overall system model and parameters

These individual component models may now be connected as indicated in figure 1, resulting in the overall control system model that can now be used to simulate the closed-loop characteristics of the TGF-β-mediated regulation of cell population. The specific model parameter values selected for the simulation studies and the details of how the parameter values are determined are presented in electronic supplementary material, section A and table S1. Briefly, most parameters were estimated from experimental observations reported in the literature (\( p_0 \), \( p_0 \) and \( r \) from Deemick et al. [15]; \( d_1 \) and \( d_2 \) from Yoo et al. [16]; \( k_{\text{deg}} \) from Hermont et al. [17]; \( k_{\text{cat1}}, K_{\text{n1}}, k_{\text{cat2}} \) and \( K_{\text{n2}} \) from Aimes & Quigley [18]; the values indicated for the controller parameters (\( K,C_a,C_b \)) were
3. RESULTS AND DISCUSSION

3.1. TGF-β-mediated regulation of normal cell growth/death

The complete control system model may now be used to simulate the dynamic regulation of the cell proliferation/death process under various conditions.

3.1.1. Nominal conditions

First, under nominal conditions indicated in electronic supplementary material, table S1, and from an initial condition of $1.0 \times 10^5$ cells in the population, the response of the overall system to a sustained step input of growth factor implemented at time $t = 0$ is shown in figure 3. Upon stimulation with growth factor, the cell number increases initially; but, as a result of an effective TGF-β-mediated controller and actuator, the cell population returns to a new steady-state value not too far from the initial value (figure 3a). The dynamics of the amount of latent TGF-β complex (LLC; controller output) and of bioactive TGF-β (actuator output) required to achieve this regulation are shown in figure 3b,c, respectively. The net result is an increase in the bioactive TGF-β level to counterbalance the effect of the sustained growth factor stimulus. Observe that under these nominal conditions, the overall system is stable (see electronic supplementary material, section B for further discussion of system stability), and the control system regulates the cell population effectively.

3.1.2. Effect of controller parameter $K$

The performance of any control system depends on the values chosen for the controller parameters. Even though this particular biological controller is nonlinear and possesses three parameters, we choose to investigate the effect of the parameter $K$ on the control system performance. This parameter can be shown to be related directly to the maximum possible controller gain, and is therefore most reminiscent of the ‘proportional gain’ value in classical feedback control. In particular, we compare the nominal controller performance to the controller performance when a ‘high $K$’ value (corresponding to twice the nominal value) is used, and also to the performance obtained when a ‘low $K$’ value (corresponding to half the nominal value) is used.

The results are shown in figure 4. Compared with nominal performance, the figure shows that a higher $K$ value results in a lower steady-state cell population, as a direct consequence of higher production of latent TGF-β; a lower $K$ value allows cells to grow more (because of the consequent lower overall production of latent TGF-β), resulting in a higher steady-state value for the cell population. In particular, taking into account that the biological controller (i.e. TGF-β producer cells) includes various types of immune cells, these results indicate that any malfunction or aberrant alterations in the immune cells’ physiology (e.g. an abnormal growth and differentiation of immune cells, reduced or delayed sensitivity to changes in target cell population, genetic and epigenetic alterations in the intracellular TGF-β production mechanism) may lead to abnormal growth of a tissue [11].

3.2. The dynamics of cancer cell population

Compared with signalling in normal cells, TGF-β signalling in cancer cells is significantly different, primarily as a result of alterations in several components of the TGF-β signalling pathway that occur during cancer progression [19]. In particular, it is known that many cancer cells, because of a variety of changes that include mutations, deletions and transcriptional downregulation, etc. express significantly fewer functional TGF-β receptors, thereby rendering them generally less responsive to TGF-β [20].
To investigate the effect of such reduced TGF-β responsiveness on the overall dynamics of TGF-β-mediated regulation in a cancer cell population, we start by observing that the cell population’s responsiveness to TGF-β is represented in the system model by the parameters $p_2$ and $d_2$, respectively, the effect of TGF-β on the proliferation rate, $p$, and the death rate, $d$. Thus, we investigate the dynamic behaviour of the TGF-β-mediated control system under cancerous conditions by comparing the performance under nominal conditions with the performance when the ‘responsiveness’ parameters $p_2$ and $d_2$, are both reduced simultaneously to 66.67, 50 and 33.33 per cent of their respective nominal values (corresponding to a 1.5-, two- and threefold reduction in responsiveness).

The simulation results shown in figure 5 display several important features. First, figure 5a (see also electronic supplementary material, figure S3) indicates that as cells become less sensitive to TGF-β, this naturally becomes less effective as a regulator of cell growth. As such, in response to growth factor stimulation, the total number of cells in the population increases, reaching progressively higher steady-state values as the individual cells become progressively less responsive. Beyond a particular point (illustrated in this case by 33.33% of nominal responsiveness), the cells would become sufficiently unresponsive—and hence sufficiently resistant to the anti-growth effects of TGF-β—to the point where the cytokine is no longer effective in suppressing unwanted proliferation. Inevitably, the cell population will therefore grow virtually indefinitely, ultimately exceeding the finite cell doubling restriction, or the so-called ‘Hayflick limit’, to which normal cells are subject [21].

These simulation results are supported by experimental observations. For example, as reported in Claus et al. [22], compared with normal prostatic cells, cells from pre-malignant prostatic tissue (i.e. benign prostatic hyperplasia) have fewer TGF-β receptors and tend to proliferate more rapidly. The signature enlargement of such glands corresponds to the higher steady-state cell population values indicated by the simulation result for cells with reduced (but stable) sensitivity to TGF-β. On the other hand, malignant prostate cancer cells (e.g. the LNCaP cell line) with their much higher proliferative potential, have even fewer (or sometimes experimentally undetectable) TGF-β receptors [23], making them much more recalcitrant to TGF-β’s tumour suppressor effects. This situation corresponds to the uncontrollable growth indicated in the simulation when the responsiveness is reduced to 33.33 per cent.

Next, the results in the other plots (figure 5b–d) show another important feature of this control system: how the ineffectiveness of TGF-β in regulating the cell population has a compounding destabilizing effect that is typical of open-loop unstable systems under ineffective feedback control. Observe that when the cell responsiveness is reduced to 33.33 per cent of the nominal value, in response to growth factor stimulation, the
cell population increases to a higher than normal level as a consequence of the lack of sensitivity to the anti-growth effect of TGF-β; the increasing cell population in turn results in continuous activation of the TGF-β synthesis machinery of the TGF-β producer cells in order to regulate the rapid abnormal growth, leading to faster attainment of the maximum level of latent TGF-β complex (LLC; figure 5b). Furthermore, the proliferating tumour cells themselves produce an increasing amount of proteases which then participate in the enzymatic activation of TGF-β, in addition to promoting the synthesis and activation of such enzymes in the microenvironment (figure 5c). The increased level of active proteases, in turn, results in rapid degradation of the ECM components, ultimately leading to increased and expedited release of bioactive TGF-β from the ECM. The increased number of cancer cells may also provide more integrins that can bind to latent TGF-β complex, thereby facilitating increased TGF-β activation by cell traction force. The net effect is shown in figure 5d where the amount of bioactive TGF-β increases exponentially in an effort to suppress runaway growth in a population of cells that have become resistant to TGF-β.

3.2.2. Stability analysis
These simulation results indicate that the cell proliferation/death process, which, from equation (2.1) is seen to be essentially ‘open-loop unstable’ [24], exhibits conditional closed-loop stability. This raises the important question: under what conditions is closed-loop stability achievable for such an open-loop unstable process? The answer is provided by nonlinear stability analysis (see electronic supplementary material, section B for the details), the primary result of which is that for this particular system, under the following mathematical condition,

$$\left( p_2 + d_2^a \right) < \left( \frac{p_0 \cdot GF^0}{p_0^e + GF^0 - d_1} \right)$$  \hspace{1cm} (3.1)$$

the system becomes structurally unstable; otherwise, the controlled process is stabilizable by feedback control. Electronic supplementary material, figure S3 shows the stability regions of the system under feedback control as a function of the parameters $p_2$ and $d_2$.

Physiologically, the left-hand side (l.h.s.) term of the stability condition in equation (3.1) represents the sum of the maximum anti-growth and death rates, while the right-hand side (r.h.s.) term represents the net intrinsic growth rate in the absence of TGF-β. Observe therefore that when the l.h.s. term is less than the r.h.s. term, the net growth rate will always be positive (i.e. $G = p - d > 0$), leading to unbounded exponential cell growth, regardless of the amount of
growth inhibitory TGF-β ligand generated and consecutively activated in the ECM.

3.2.3. Implications

Taken together, these simulation and stability analysis results reveal a new paradigm for understanding of the TGF-β paradox as it now appears evident that under the control system postulate investigated above, the observed increased level of TGF-β is a consequence of the acquired TGF-β resistance exhibited by the cancer cells, not the cause. As such, the correlation between the increased level of TGF-β and poor prognosis, while real, should not have been interpreted as implying that the former caused the latter. The implications are that as pre-malignant cells lose their responsiveness to TGF-β along the spectrum of tumour progression, a still-intact control system must secrete more of this cytokine in a futile attempt to achieve the same level of tumour suppression attainable with normal, responsive cells. (On a single-cell level, this is consistent with our previous mathematical model of intracellular TGF-β signalling via Smad [6] which showed, among other things, that the amount of TGF-β needed to produce a saturated Smad-mediated response in a cancer cell is far higher than that in healthy cells, implying that to elicit nuclear Smad-mediated (growth inhibitory) activity, cancer cells require more TGF-β than normal.) Our control system model therefore indicates that there is no paradox: TGF-β remains a tumour suppressor; and observing increased levels of TGF-β in poor prognosis patients is entirely consistent with TGF-β’s role as a tumour suppressor which attempts to regulate the growth of aberrant cells that have already lost their sensitivity to the cytokine.

4. CONCLUSIONS

We have studied the role of TGF-β in normal and cancerous cells using a control engineering model of TGF-β-mediated regulation of cell population dynamics. In particular, the results of our study indicate that the correlation between increased levels of TGF-β and poor prognosis may have been inadvertently misconstrued as causality, creating an apparent paradox. Our results indicate that the clinically observed increase in TGF-β levels in cancerous tissues and serum is not an indication that the tumour suppressor role of TGF-β has changed fundamentally. Rather, the control system perspective supports the hypothesis that the role of TGF-β as a tumour suppressor is unchanged, and further stipulates that the level of TGF-β should in fact increase in an attempt to elicit normal responses from a tumour that is becoming increasingly resistant to this cytokine. Thus, the clinical observations are actually consistent with a TGF-β whose role as a tumour suppressor remains unchanged.

A key next step is to validate this hypothesis experimentally, in vitro, using the following approach. Several cancer cell lines along the spectrum of cancer progression from normal to highly malignant, whose functional TGF-β receptor levels are well characterized, will be stimulated with identical amounts of growth factors and allowed to begin proliferating. Measured amounts of TGF-β will then be added to each growing population progressively until growth is arrested. The amount of TGF-β required to suppress growth completely will then be noted for each cell line. If the hypothesis is true, it is expected that higher amounts of TGF-β will be required to suppress growth completely for the more malignant cell lines.

If this control systems hypothesis is confirmed, the consequences for how TGF-β ligand and TGF-β receptors are used as therapeutic agents could be significant. Specifically, it will mean that the current approach of targeting TGF-β ligand therapeutically (e.g. removal or deactivation of TGF-β in cancer tissues) may have to be abandoned in favour of re-sensitizing the cells to the tumour suppressive effect of the TGF-β, similar to treatment for diabetes mediated by prolonged insulin-resistance [25]. In fact, this statement is consistent with experimental findings that restoration of TGF-β receptor (type I and/or type II) expression results in growth inhibition in a variety of cancer cells (e.g. prostate [26], pancreatic [27], colon [28], lung [29] and several other cancer types). Therefore, the results presented here suggest that therapy based on full recovery of TGF-β signalling in cancer cells through either gain of function (e.g. gene insertion/replacement via gene therapy) and/or loss of function (via removal or enervation of TGF-β-signalling inhibitors) should be given serious consideration as a viable alternative cancer treatment strategy in cases where TGF-β has been implicated.

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