Mathematical model of the role of intercellular signalling in intercellular cooperation during tumorigenesis

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Abstract

Objectives: Intercellular cooperation has been hypothesized to enhance cell proliferation during cancer metastasis through autocrine signalling cascades and mathematical models can provide valuable insights into underlying mechanisms of metastatic tumorigenesis. Here, we present a model that incorporates signal-stimulated cell proliferation, and investigate influences of diffusion-driven heterogeneity in signal concentration on proliferation dynamics.

Materials and methods: Our model incorporates signal production through both autocrine and paracrine pathways, and signal diffusion and loss for a metastasizing cell population at a host site. We use the signalling pathway of IL-6 for illustration where this signalling species forms an intermediate complex with its receptor IL-6R. This in turn forms a heterodimeric complex with transmembrane protein gp130, ultimately resulting in production of downstream signals. Cell population dynamics are taken to follow a modified logistic equation for which the rate term is dependent on local IL-6 concentration.

Results and conclusions: Our spatiotemporal model agrees closely with experimental results. The model is also able to predict two phenomena typical of metastatic tumorigenesis – host tissue preference and long periods of proliferation dormancy. It confirms that diffusivity of the signalling species in a host tissue plays a significant role during the process. Our results show that the proliferation–apoptosis balance is tipped in favour of the former for

host sites that have relatively smaller signal diffusivities.

Introduction

Intercellular cooperation among unicellular organisms leads to community behaviour, for example through biofilm formation by bacteria (1), and can also enhance cell proliferation and resistance to therapy during disease (2). A key basis for intercellular cooperation is that cells are able to ascertain their local population densities (3–5). Community behaviour during tumorigenesis has long been hypothesized for metastasizing cancer cells (2) and recent literature provides evidence for roles of autocrine signalling cascades during tumorigenesis (6), particularly for lung (7) and breast (8) malignancies. Mathematical models of tumorigenesis incorporating intercellular communication were first reported in 1997 (9). Although there are several empirical (10–12) and mechanistic models for both upstream (extracellular) (6) and downstream (intracellular) (13) signalling cascades, both types are based on temporal changes. Up to now, there have been no reports of mathematical investigations that have modelled and explained the influence of diffusion-driven spatial heterogeneity of signalling species on tumorigenesis, which is our focus herein.

Investigations of intercellular signalling during tumorigenesis point to IL-6 (6–8,14), a soluble cytokine, as a signalling species responsible for aggressive cell proliferation, that is characteristic of many tumours. IL-6 molecules present in tumour microenvironments can be generated by two pathways. The autocrine pathway is dominant where tumour cells are themselves responsible for producing the signalling species and the paracrine pathway involves signalling molecules generated by host tissue cells, typically augmenting autocrine signalling. Certain experimentation has provided evidence of higher IL-6 mRNA expression in mammospheres from

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node-invasive breast carcinoma tissues than from matched non-neoplastic mammary glands, and have shown that IL-6 from both autocrine and exogenous sources accelerates self-renewal of mammospheres (8). Reduction in tumorigenesis was found when IL-6 levels were lowered by blocking autocrine pathways that lead to its production (7). Therefore, we hypothesize that proliferation of metastasizing cells is dependent on local IL-6 concentration during tumorigenesis.

We present a model for a spherically symmetric volume containing a metastasizing cell population, that incorporates both signal production through autocrine pathways, signal loss (for example due to blood perfusion) (15,16), and signal consumption. This general model explains tumorigenesis and provides insight into secondary tumour growth from metastasizing cancer cells. Its predictions are consistent with reported observations of metastatic growth. Certain tumour cells are known to show definite predilection for one or more specific host sites and our results indicate that this 'seed to soil' organ preference of metastasizing cells (17–19) can be explained in part through intercellular signalling. The model also predicts the long dormancy of isolated cells (20) with a sharper 'wake up' than conventional models, which do not consider intercellular cooperation. Despite experimental evidence for several other factors [for example, matrix metalloproteinases (20), that govern cancer cell proliferation rates], we restrict our model to a single signalling species for the sake of simplicity, as the model can be enhanced later without any loss of generality. The model provides general understanding of the role of diffusion in signalling and can be extended to accommodate several independent or coupled autocrine or paracrine signalling pathways responsible for tumorigenesis.

The model

The signalling pathway for IL-6 in our model is illustrated in Fig. 1. The signal S , that is IL-6, forms an intermediate complex X_1 with its receptor IL-6R. This in turn, forms a heterodimeric complex with the transmembrane protein gp130, which we denote as T , ultimately resulting in downstream signals (6,21) that are denoted by P . These pathways are represented through the reactions (22),

Equation 1 represents the overall pathway (IL-6 + IL-6R) $\leftrightarrow$ intermediate complex, and (gp130 + intermediate complex) $\leftrightarrow$ proliferation signals. The signals depend upon production of an intermediate chemical species,

Figure 1. IL-6 signal molecules originate in the tumour microenvironment from both autocrine (similar tumour cells) and paracrine (host tissue cells) sources. IL-6 forms a complex (X_1) with its receptor (IL-6R), and this subsequently forms a complex (X_2) with the transmembrane protein gp130 (T), releasing the intracellular signalling species P . The latter enhances the cell proliferation rate and autocrine signal production.

denoted generically as X_2 . With number of receptors $\rho \propto N$ where N denotes cell population density, signal consumption rate due to reactions represented in eqn (1),

$$\frac{ds}{dt} = \frac{-(k_3 \rho s^2)}{((k_{-1}/k_1)((k_3 + k_{-2})/k_2) + s^2)}. \quad (2a)$$

When $k_2 (k_3 + k_{-2}) > k_1/k_{-1}$, forward rate of the first reaction along the pathway (1b) proceeds far more rapidly than the corresponding forward rate of reaction (1a) (22). For this to occur, the intermediate complex must dissociate slowly in comparison with its production. The model assumes that signal consumption initiates intercellular cooperation, for example, through gene expression. Thus, above a threshold signal consumption rate $(ds/dt)_c$, individual cells behave as a community. A more general form of eqn (2a) is

$$\frac{ds}{dt} = \frac{-N\alpha s^2}{(\beta + s^2)} \quad (2b)$$

with α and β as functions of the biomolecular kinetics, and α being further dependent on number of receptors per cell. Introducing one-dimensional Fickian diffusion, the spatiotemporal distribution of s ,

$$\frac{\partial s}{\partial t} = D \frac{\partial}{\partial r} \left(r^2 \frac{\partial s}{\partial r} \right) + N(r, t) \left(q(s) - \frac{\alpha s^2}{\beta + s^2} \right), \quad (3)$$

where D denotes signal diffusivity, q a signal production term and $N(r, t)$ local cell population density at any time. The symbols and parameters are explained in detail in Tables 1 and 2.

Table 1. Nomenclature

Symbol	Nomenclature	Unit
$N(r,t)$	Local cell population density	cells cm^{-3}
r	Radial distance from the centre of tumour	cm
$s(r,t)$	Local signal (IL-6) concentration	ng cm^{-3}
t	Time	days

Cell population dynamics are taken to follow the modified logistic equation

$$\frac{dN(r,t)}{dt} = p(s)N(r,t) \left(1 - \frac{N(r,t)}{B}\right), \quad (4)$$

where rate term $p(s)$ is dependent on local IL-6 concentration. Here B denotes maximum carrying capacity of the environment, imposed by constraints such as nutrient supply and cell size.

The volume housing these cells is assumed to be spherically symmetric. Due to symmetry, $\partial s / \partial r_{r=0} = 0$, there is no signal flux across the centre of the sphere. We hypothesize that the rate parameter $p(s)$ is linearly related to signal consumption rate in eqn (2),

$$p(s) = p_0 + \phi \frac{\alpha s^2}{\beta + s^2}, \quad (5)$$

where p_0 is a constant term characteristic of inherent proliferation rate of the cell that is independent of external factors, and ϕ is a constant which relates reaction kinetics in the signal transduction pathway to proliferation dynam-

ics, which is likely to be dependent upon number of receptors per cell amongst other factors.

While the literature (23–25) indicates that autocrine IL-6 production rate of oestrogen receptor (ER)- α -positive cell lines (for example, MCF-7, BT474) is negligibly small in culture medium, these studies have typically involved a culture medium without any exposure to recombinant IL-6. High levels of IL-6 mRNA have been reported when mammospheres comprising MCF-7-derived cell lines were exposed to IL-6 (8,26), suggesting that IL-6 upregulates its own mRNA in a positive feedback loop. Hence, it is reasonable to assume a linear relationship between IL-6 production and consumption rates of a particular cell type, that is,

$$q(s) = q_0 + \psi \frac{\alpha s^2}{\beta + s^2}, \quad (6)$$

where the term q_0 is characteristic IL-6 production rate of the cell type independent of external factors. Thus, $q_0 = 0$ for all ER- α -positive cell lines (for example, MCF-7, BT474), and non-zero for ER- α -negative lines (for example, MDA-MB-468) (23–25,27). Equation (3) can therefore be written as

$$\frac{\partial s}{\partial t} = \frac{D}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial s}{\partial r} \right) + N(r,t) \left(q_0 + \xi \frac{\alpha s^2}{\beta + s^2} \right), \quad (7)$$

where $\xi = \psi - 1$. Coupled eqns (4) and (7) are solved for specified initial and boundary conditions using the numerical solver *NDSolve* in Mathematica[®]. Parameter values used in the solution are detailed in Table 2.

Table 2. Parameters

Symbol	Parameter	Value ^a	Units
B	Maximum cell population density	10^7	cells cm^{-3}
D	Diffusivity	10^{-4}	$\text{cm}^2 \text{ day}^{-1}$
N_0	Initial cell population density	10^5	cells cm^{-3}
p_0	Constant term in rate parameter $p(s)$ of the modified logistic equation, defined in eqn (5)	5×10^{-2}	day^{-1}
q_0	Constant part of the signal generation term, defined in eqn (6)	ER- α -positive: 0 ER- α -negative: 10^{-6}	ng cell $^{-1}$ cm^{-3} day^{-1}
R	Radius of tumour	0.1	cm
s'	Characteristic signal concentration	10^2	ng cm^{-3}
α	Signal consumption rate parameter, defined in eqn (2b). Order of magnitude of $\phi\alpha$ from reference (23).	5×10^{-7}	ng cell $^{-1}$ day^{-1}
β	Signal consumption parameter, defined in eqn (2b). From reference (23)	233.2	ng 2 cm^{-6}
ϕ	Responsiveness of proliferation rate parameter $p(s)$ on signal consumption rate, defined in eqn (5)	10^5	cells ng $^{-1}$
ψ	Order of magnitude of $\phi\alpha$ from reference (23). Responsiveness of signal generation rate per cell [$q(s)$] on signal consumption rate, defined in eqn (6)	5	dimensionless
ξ	$\psi - 1$	4	dimensionless
τ	Characteristic time scale	10^2	days
γ	Rate parameter in conventional logistic equation	0.05	day^{-1}

^aUnless otherwise specified.

Model validation and parameter estimation

We determined parameters $\phi\alpha$ and β by fitting growth curves generated by eqns (4) and (5) to the experimental data for two breast cancer cell lines MCF-7 and BT474 that are presented in Fig. 2a,b of reference (23). Cell population growth curves shown in those figures are for different doses of recombinant IL-6. We have extracted those data using plot digitization software. An exhaustive search of variations in the solution of eqn (4) within a specified range of the parameter $p(s)$ provides the best possible value for a particular cell line and signal concentration. This best fit is corroborated by coefficient of determination R^2 that lies closest to unity (see Fig. 2a,b). The best fit for each data set indicates that carrying capacity $B = 8N_0$ fits all of the data satisfactorily for which the minimum coefficient of determination $R^2 = 0.9515$.

The set of values of $p(s)$ for a particular cell line at different IL-6 concentrations subsequently forms the basis for an exhaustive search for the best possible values of $\phi\alpha$ and β , as shown in Fig. 2c,d. This is again accomplished by determining R^2 value closest to unity for which $p(s)$ is obtained using eqn (5). While biochemical species

involved in the signalling pathways for both cell lines are the same, the value of $\phi\alpha$ applicable to each can differ because α is proportional to number of receptors per cell. However, using the β value obtained for MCF-7 cell line (in Fig. 2c) to predict the growth rate parameter for the BT474 cell line (in Fig. 2d) provides an excellent fit with $R^2 = 0.9933$. This further corroborates the hypothesis on which eqn (5) is based, that is that $p(s)$ is linearly related to signal consumption rate.

For additional proof of the hypothesis, we used these parameters to predict resultant cell population concentration for a wider range of IL-6 doses at the end of day 8, from data presented in Fig. 2c of reference (23). This also results in satisfactory fits to the experimental data (with $R^2 = 0.9529$ for MCF-7 and $R^2 = 0.927$ for BT474). The only data available for IL-6 production are for evolution of concentration of this species in culture medium containing MCF-7/ADR cell line (25). However, as corresponding population dynamics are unavailable, validating dependence of IL-6 production on signal concentration in the context of eqn (6) is left as a future exercise.

We used the model to predict spatiotemporal evolution of local cell population density from eqn (4) for initial

Figure 2. The rate parameter $p(s)$ in eqn (5) changes with the signal concentration s for both MCF-7 (a) and BT474 (b) cell lines, as determined from the population growth curves in Ref. (23). The rate parameter $p(s)$ determined from the signal concentration-dependent variation in the growth rate fits the hypothesized eqn (5) for both MCF-7 (c) and BT474 (d), and the parameters $\phi\alpha$ and β are subsequently determined from an extensive search within relevant ranges for the maximum value of R^2 .

conditions $N_{t=0} = 10^5 \text{ cells cm}^{-3}$, and $B = 10^7 \text{ cells cm}^{-3}$. As the characteristic dimension of a cell is considerably smaller than tumour radius $R = 0.1 \text{ cm}$, spatiotemporal signal distribution follows a continuum rather than occurring in discrete steps. We assumed that $D \approx 10^{-4} \text{ cm}^2 \text{ day}^{-1}$. This is based on characteristic diffusion of a lysozyme molecule in agarose gel (28) due to similarity of its Stokes radius (29) with that of the IL-6/gp130 complex, which provides a rough estimate of the order of magnitude of required diffusivity. We recognize that results obtained using this value of diffusivity do not provide quantitatively accurate predictions, however, as there is lack of available data for several relevant parameters in the model, an accurate value of diffusivity is of little significance. This rough estimate provides a general insight into the role of diffusion of the signal species.

Kinetic rate parameters associated with the entire signalling pathway are not available in the literature. Thus, to obtain a quantitative understanding of the problem, we selected some parameters based on conditions adopted in the experimental procedure of reference (23). Other parameters are determined on a mathematical basis, that is, by ensuring that each term in eqns (4) and (7) is significant in solutions to these equations. We assumed that $N(t) \sim B \approx O(10^7) \text{ cells cm}^{-3}$ and $s \sim s' \approx O(10^2) \text{ ng cm}^{-3}$, where s' is a characteristic signal concentration, $\beta = 233.2 \text{ ng}^2 \text{ cm}^{-6}$ and $\phi\alpha \sim O(10^{-1}) \text{ day}^{-1}$, which are all values consistent with experimental observations.

With orders of magnitude for time $\tau \sim 100$ days and radius $r \sim R (\sim 0.1 \text{ cm})$, $\partial s / \partial t \sim (s' / \tau)$ and $(D / r^2)(\partial / \partial r)(r^2 \partial s / \partial r) \sim (Ds' / R^2)$ are both of $O(10^0) \text{ ng cm}^{-3} \text{ day}^{-1}$. The other terms in eqn (7), $(N(r,t)q_0 \sim Bq_0) \approx (N(r,t)((\xi\alpha s^2)/(\beta + s^2)) \sim B((\xi\alpha s'^2)/(\beta + s'^2))) \approx O(10^0) \text{ cells cm}^{-3} \text{ day}^{-1}$, i.e. $q_0 \approx O(10^{-7}) \text{ ng cell}^{-1} \text{ cm}^{-3} \text{ d}^{-1}$ and $\alpha \approx O(10^{-7}) \text{ ng cell}^{-1} \text{ day}^{-1}$. In addition, from eqn (4), $dN(r,t)/dt \sim (B/\tau) \approx O(10^5) \text{ cell cm}^{-3} \text{ day}^{-1}$. Thus the terms on the right hand side in Eq. (4), $(N(r,t)p_0(1 - N(r,t)/B) \sim Bp_0) \approx (N(r,t)\phi\alpha s^2/(\beta + s^2) \sim B\phi\alpha) \approx O(10^5) \text{ cell cm}^{-3} \text{ day}^{-1}$, i.e. $p_0 \approx O(10^{-2})$ and $\phi \approx O(10^5) \text{ cell ng}^{-1}$, the latter of which is consistent with the order of magnitude encountered for ϕ in the experimental data.

Results and discussion

The model is used to predict cell proliferation patterns in two cell types – ER- α -positive and -negative cells, over a time interval relevant for tumorigenesis. It helps to illustrate evolution of spatially heterogeneous population density at a metastatic site for a range of relevant conditions. Results contradict previously reported inferences concerning lack of sensitivity of growth rate of ER- α -negative

cells (for example, MDA-MB-231, MDA-MB-468) to local IL-6 concentration (23,24,27). The model is able to predict two phenomena that are typical of metastatic tumorigenesis – host tissue preference and long periods of dormancy. We also investigated impact of possible therapies targeted specifically at this signalling pathway.

Proliferation behaviours

Figure 3 presents a comparative depiction of evolution of spatiotemporal cell and signal concentration distributions for ER- α -positive and -negative cell types. For physical relevance, we selected an initial and boundary signal concentration of 3 ng cm^{-3} , which is consistent with levels observed in cultured bone and breast fibroblasts (23). Local cell proliferation rate parameter $p(s)$ increases over time due to increase in local signal concentration that is caused by autocrine signal production. The larger the number of cells, the greater the signal that is produced, resulting in larger values of $p(s)$, which would ultimately reach saturation.

To contrast spatiotemporal growth patterns shown in Fig. 3a,b that are caused solely by differences in IL-6 production characteristics of the two cell types, we kept all parameters other than q_0 equal in the two simulations. ER- α -negative cells produce IL-6 even in the absence of paracrine sources, which is modelled by setting $q_0 \neq 0$ in eqn (6). Signal generation rate is augmented by presence of a paracrine signal. This explains the higher signal concentration in a cluster of these cells compared to ER- α -positive variants, as shown in Fig. 3c,d. Thus, any metastatic site hosting ER- α -negative cell lines would initially have higher growth rates than ER- α -positive cell lines at the same site. The latter cell type initially experiences slower population growth, but one that rises sharply when signal concentration ultimately increases, as shown in Fig. 3e,f. This is also observed in experimental investigations for MDA-MB-231 and MCF-7, data for which are presented in Fig. 3a,c of reference (27).

Dependence of proliferation rate on IL-6 concentration

Cell proliferation rate of ER- α -negative cells has been reported to be significantly less responsive to local IL-6 concentration than that of ER- α -positive cells (23,24,27). This claim is based on experimental observations in culture media provided with different initial doses of recombinant IL-6. Experimental measurements show that there are differences in population growth response to signal concentration even among the same type of cells [for example, please note difference in $p(s)$ for MCF-7 and BT474 cells based on reference (23), as discussed earlier]. This may be explained by differences in number of

receptors per cell, which affects signal consumption rate. However, the characteristic difference in responsiveness can also be linked to receptor expression and different signal production properties. Signal consumption kinetics represented in eqn (1a,b) establish signal concentration s_t beyond which local consumption rate is saturated. Linear dependence of growth parameter $p(s)$ on consumption rate assumed in eqn (5) implies that $p(s)$ also saturates beyond s_t , as shown in Fig. 4a. Figure 4b shows that evolution of signal concentration depends significantly on paracrine signal sources for ER- α -positive cells, but that this dependence is much more limited for ER- α -negative cells. This is explained by ability of the latter cells to produce IL-6 even when no paracrine sources exist. As autocrine signal generation rate in this type is inherently larger, cell clusters quickly reach threshold signal concentration and thereby saturate $p(s)$ sooner. Any subsequent

proliferation is unaffected by further increase in signal concentration, either through addition of recombinant IL-6 or any other means, as the local signal concentration exceeds s_t .

We present two simulations for population dynamics for which parameters relating $p(s)$ to signal concentration (ϕ , α and β) are identical for both ER- α -positive and -negative cell types. As discussed above, ER- α -negative cells are less responsive to paracrine IL-6 sources than ER- α -positive variants, as illustrated through Fig. 4c,d. While inflexions in population growth curves for ER- α -negative cell types lie closer together for different signal concentrations, these occur further apart for the positive cell type. Population dynamics presented in Fig. 4e,f confirm our hypothesis, thus, despite the two simulated populations having similar dependence on local IL-6 concentration, their proliferation dynamics

Figure 3. The radial distribution of cell population densities of ER- α -negative (a) and ER- α -positive (b) cells at specific instants shows that ER- α -negative cells typically have a higher population density at any instant during the proliferation phase. This is due to the typically larger local signal concentration in a cluster of ER- α -negative cells (c), as opposed to the ER- α -positive variants (d) because they produce autocrine signal even in the absence of paracrine signal sources [$q_0 \neq 0$ in eqn (6)]. Thus, (e) the ER- α -negative cells proliferate more aggressively than the ER- α -positive lines, (f) which have enhanced proliferation once the signal concentration catches up.

differ. Therefore, a more accurate statement in this respect is that growth rate of ER- α -negative cells is less responsive to paracrine IL-6 sources than that of ER- α -positive cells, although it is related similarly to the local IL-6 concentration.

Tumour cell proliferation rate is controlled by IL-6 signalling through activation of the transcription factor signal transducer and activator of transcription 3 (STAT3) (6,24). An experimental study by Sasser *et al.* (24) showed low basal activation of STAT3 in ER- α -positive cells until they were exposed to IL-6. ER- α -negative cells, however, had high basal activation level of STAT3 and exposure to IL-6 did not influence them significantly. This saturation in activation above a particular concentration of IL-6 agrees with population dynamics predicted through our model, as shown in Fig. 4e,f.

Dormancy of metastasizing cells

Cancer cells at secondary sites can remain dormant for long periods before further active proliferation occurs (30,31), sometimes even for over a decade. Cell populations have been known to undergo sudden wake-up followed by aggressive proliferation (32). Some investigators have enabled dormancy in cell populations by balancing proliferation and apoptotic rates, using angiogenic inhibitors (33,34). However, there is no evidence of such activity at real metastatic sites, neither does reduced apoptosis explain rapid proliferation rates detected in clinical cases. A competing theory explains periods of dormancy in terms of low cell concentration at secondary sites (35,36) that are linked to presence of solitary cells in the tissue (37).

Figure 4. Differences in IL-6 production characteristics significantly alter population dynamics in the two cell types. (a) The rate parameter $p(s)$ saturates beyond a threshold signal concentration [eqn (5)]. (b) The signal concentration at the centre of the tumour, i.e. $s(0,t)$ is highly dependent on the paracrine signal sources for ER- α -positive cells. This has significant impact on differences in the evolution of the proliferation rates of (c) ER- α -negative and (d) positive cell types so that the population dynamics of (e) the ER- α -negative cells are less responsive to paracrine signal sources than of (f) ER- α -positive cells. The results have been generated using the same values for all parameters that relate the dependence of $p(s)$ to the local IL-6 concentration for the two cell types.

To explore ability of our model to predict such periods of dormancy, we compared results with the dynamics predicted by a conventional logistic equation

$$\frac{dN}{dt} = \gamma N \left(1 - \frac{N}{B} \right) \quad (8)$$

for which average rate parameter γ is set as half of the maximum value of $p(s)$ for that cell type. Figure 5a contrasts evolution of the cell population determined from eqns (4) and (7) at the centre $r = 0$ of a spherical tumour, with results obtained from eqn (8). The spatiotemporal model predicts a more sudden wake-up, which is closer to observations made during tumorigenesis (32), compared to logistic relations. This occurs because, as signal concentration increases, the rate parameter $p(s)$ also increases, increasing the cell proliferation rate. Consequently, a vigorous growth phase ensues that lasts for shorter duration as cell population density quickly saturates, which then causes cell proliferation to cease. The growth phase predicted by the logistic equation is, however, more gradual in comparison and peak proliferation rate reached is lower than that predicted by the spatiotemporal model, as illustrated in Fig. 5b.

We next focused on influence of variations in initial cell population on population dynamics. Figure 5c presents evolution of cell population density at the centre of a

spherical tumour for two initial populations. Assuming that clinical investigations reveal onset of tumours only when cell population reaches close to 10^6 cells cm^{-3} , a tumour with initial population density $N_0 = 10^5$ cells cm^{-3} would be detected almost immediately after it is allowed to proliferate, whereas one with lower initial population of 10^2 cells cm^{-3} would be detected only after ≈ 175 days. Although the logistic equation predicts similar results, it is apparent that the delay between results for the two initial conditions is longer for the spatiotemporal model. Furthermore, associated wake-up is more abrupt due to the influence of signal consumption rate in the spatiotemporal cell proliferation relation of eqn (4).

Seed to soil effects in metastasis

Some cancer cells are known to exhibit organ-specific preferences for metastatic growth (17). As haematogenous pathways are the main mode of cell transfer for these phenotypes, it is intuitive to assume that transfer along these paths is primarily dependent on blood circulation network downstream of the primary site. This is supported by high efficiency of physical arrest of cancer cells by certain organs due to characteristic dimensions of fluid transport networks within them (17,38,39). For instance, as cancer cells (which have a average diameter of $7 \mu\text{m}$) are

Figure 5. (a) The modified logistic equation [eqn (4)] produces a more abrupt increase in the cell population density than does the conventional logistic equation with a rate parameter $\gamma = 0.05$, which is half of the maximum value attained by $p(s)$ for the other simulations presented in panel. (b) The peak cell proliferation rate predicted by the modified form of the equation is larger and the proliferation phase is narrower. (c) A site with an initial population of $N_0 = 10^5$ cells cm^{-3} attains peak proliferation rate much sooner than the one that begins with $N_0 = 10^2$ cells cm^{-3} . Such a site also saturates much sooner after the inception of metastasis. (d) The peak proliferation rate achieved is the same in either case.

comparatively larger than the capillaries through which they might move (which themselves may have dimensions of only 3–8 μm) large proportions of these cells are likely to be arrested by the first capillary bed that they encounter. Arrest can also involve chemokine receptors on cancer cells that allow them to home to specific organs (17). In either case, a small proportion of the cells that leave the primary site ultimately produce a successful metastatic site.

Recent investigations indicate that preference of a metastasizing cell for a secondary site is primarily regulated by efficient organ-specific population growth and not high degree of arrest (17,40–42). According to these studies (40–42), molecular interactions with the tumour microenvironment in the host tissue play a key role in enabling successful metastasis. This is primarily manifested through molecular interactions between many chemical species. While interactions responsible for such growth regulation are not yet fully understood, we have modelled the role of a single signalling species, which acts as a growth regulator in the tumour microenvironment, such as IL-6 (23,24,43); however, it should be noted here that IL-6 is only one of several factors at play. Exhaustive exploration of all factors should be very specific to a cancer type and is therefore well beyond the scope of this paper. Our results simply illustrate the role of host site

diffusivity in tumour growth for a hypothetical cell line for which the proliferation kinetics are solely governed by local IL-6 concentration, as modelled by eqns (4) and (5).

Fibroblasts isolated from bone and lung, two competing metastatic sites for breast cancer, show comparable levels of IL-6 (23). As these results are from an experiment in an isolated culture medium, this would mean that they have similar signal production rates. However, ER- α -positive cells show particular predilection for bone despite low probability of physical transport of cancer cells from the primary site (17). Differences in signal diffusivities in different organs would lead to dissimilar concentration distributions of signalling species at any instance, and we hypothesized that this can explain affinity for bone. Bones typically have lower diffusivity than several other organs which lie in the immediate vicinity of primary sites of breast cancer and enjoy a higher proportion of arrested cells. Thus, rate of increase in IL-6 concentration in bones is also much larger than at other sites, leading to accelerated growth, as illustrated in Fig. 6. Figure 6a presents evolution of signal concentration at the centre of the tumour for two different diffusivities ($D = 10^{-4} \text{ cm}^2 \text{ day}^{-1}$, $D = 0.5 \times 10^{-4} \text{ cm}^2 \text{ day}^{-1}$) for ER- α -positive cells. Resulting population dynamics presented in Fig. 6b clearly shows that more rapid cell proliferation occurs in a lower diffusivity medium. Figure 5c,d show the radial

Figure 6. Lower diffusivity lowers the loss of IL-6 by diffusion, accelerating local signal concentration growth. (a) The steady-state signal concentration at the centre of the tumour doubles if the diffusivity is halved. (b) This causes cell population to attain a higher proliferation in a medium of lower diffusivity at any instant until the population density comes close to saturation. (c) The signal concentration distribution 75 days after the inception of metastasis shows that the signal concentration at the centre of the tumour increases almost 5-fold if the diffusivity be halved. (d) The corresponding cell population density is almost twice that obtained in the medium with higher diffusivity.

distributions of the signal concentration and cell population after 75 days from the beginning of metastasis. As expected, the cell population density in the low diffusivity medium reaches almost the double of the peak population in the high diffusivity medium. Although our model does not incorporate apoptosis and necrosis, we argue that the proliferation–apoptosis balance is tipped in favour of the former for host sites that have relatively smaller signal diffusivity.

Cancer therapy through receptor blocking

Based on the significance of intercellular signalling on tumorigenesis that has been illustrated above, we investigate the efficacy of a therapy to curb tumour cell proliferation rates by blocking IL-6 receptors. Sasser *et al.* (24) have reported that neutralizing the receptors on ER- α -positive cells allowed inhibition of the ability of human mesenchymal stem cells to induce STAT3 phosphorylation, thereby checking cell proliferation. The degree of inhibition depended on the concentration of the neutralizing antibodies used in the experiment.

The parameter α is proportional to the number of receptors present per cell. Thus, the dynamics of a cell population under therapy can be predicted by reducing α by a factor, which equals the percentage of receptors blocked successfully. Figure 7a compares the population dynamics of an untreated metastatic site with that of a similar site under therapy. For a particular phenotype with $\alpha = 5 \times 10^{-7} \text{ ng cell}^{-1} \text{ cm}^{-3} \text{ day}^{-1}$, blocking 90% of receptors would reduce α to $5 \times 10^{-8} \text{ ng cell}^{-1} \text{ cm}^{-3} \text{ day}^{-1}$, and if all receptors are successfully blocked off, $\alpha = 0$. Both these cases significantly hinder cell proliferation. The peak proliferation rate is almost halved if 90% of the receptors are blocked off and the corresponding peak proliferation is reached almost 30 days later than when $\alpha = 5 \times 10^{-7} \text{ ng cell}^{-1} \text{ cm}^{-3} \text{ day}^{-1}$ (see Fig. 7b). Thus, a therapy targeted at receptor blocking could be successful in augmenting conventional chemotherapeutics, as lowering the rate required to remove tumour cells translates into a lower dosage for a drug. Consequently, the damage caused to healthy cells can also be lowered.

Conclusion

Recent studies have established autocrine signalling as the basis for quorum sensing during tumorigenesis. Conventional models of cell population dynamics must therefore be modified to account for the signal-stimulated cell proliferation observed in such multicellular communities. We address this requirement by incorporating the local signal concentration in the population dynamics by modifying the logistic equation. As autocrine signal species tend to

Figure 7. Receptor blocking can aid conventional cancer therapies in checking tumor growth. (a) Blocking a part or all of the IL-6 receptors slows down tumour growth. (b) The proliferation rate is significantly inhibited and is related to the percentage of blocked receptors and the abruptness of the proliferation is significantly reduced, potentially augmenting the efficacy of other therapies.

diffuse out of a tumour, this leads to a spatial heterogeneity in the signal concentration, which induces spatially non-uniform proliferation rates that are unaccounted for in conventional models. Herein, the generation, consumption, loss and diffusion of the signalling species are modelled for an idealized spherically symmetric cell cluster with a dynamically growing cancer cell population. Reported experimental data are used to determine parameter values associated with the model and to provide general proof of the concept. The model predicts some characteristic behaviours observed during metastasized cancer cell proliferation. Lowering the initial population concentration of the cells by three orders of magnitude increases the time before clinical detection of a metastasized tumour can occur by an order of magnitude, much in the manner that dormancy is observed for isolated metastasized cells at secondary sites. The model also predicts the sudden wake up that follows a period of dormancy when rapid proliferation occurs. We further hypothesize that the diffusivity of the signalling species in a host organ plays a role in the seed to soil preferences observed during metastasis. This is predicted through our model when, by simply halving the signal diffusivity at the site, we are

able to increase the local proliferation rate so that the resultant cell population at a particular location almost doubles after just 75 days. The results support our contention that the spatial diffusion of autocrine signals plays a vital role in intercellular cooperation and cellular proliferation during tumorigenesis. The model also illustrates a contradiction on the reported lack of responsiveness of ER- α -negative cells on the local IL-6 concentration compared with the ER- α -positive cells and instead points to the lack of responsiveness to paracrine IL-6 sources. The significance of autocrine signalling naturally suggests therapies to inhibit the associated pathways. We use the model to investigate the efficacy of receptor blocking as a therapy to augment conventional chemotherapies by significantly reducing the required dosage. This reduces the unwanted destruction of the surrounding healthy cells. While further validation with *in-vivo* data is required, the model provides mathematical insight into signalling-induced tumorigenesis and the various factors that govern the process.

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