Supporting Material

miR451 and AMPK mutual antagonism in glioma cell migration and proliferation : A mathematical model

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S1. Detailed description of the models and parameters used.

Parameter estimation

Diffusion coefficients : D_n, D_P, D_G

D_n : For EC cells migrating in a culture containing angiogenic factor, Stokes and Lauffenburger [1] measured the diffusion coefficient to be 7×10^{-9} cm^2/s. A 'typical' animal's cell motility coefficient has been estimated to be 5×10^{-10} cm^2/s [2]. A smaller value, 10^{-10} cm^2/s, was used in [3]. While the experimental results for motility of human glioma [4] and glioblastoma cells [5] in 2D substrate suggest a value of D_n in the range of 1.16×10^{-10}-2.31×10^{-9} cm^2/s, Stein *et al.* [6] used a 10-fold higher value of D_n ($=2.31\times10^{-8}$ cm^2/s) in order to get a better fit to the experimental data. Burgess *et al.* [7] took $D_n=1.7 \times 10^{-9}$ cm^2/s, but Sander and Deisboeck [8] argued that D_n should be much smaller, namely, 10^{-12} cm^2/s. We shall take $D_n=10^{-11}$ cm^2/s.

D_P : In experiments of the movement of MMP-1 in the collagen fibril, Saffarian *et al.* [9] estimated the diffusion coefficient to be $(8\pm1.5)\times10^{-9}$ cm^2/s for wild-type activated MMP-1 and $(6.7\pm1.5)\times10^{-9}$ cm^2/s for inactive mutant. In our simulation, we take $D_P = 5 \times 10^{-11}$ cm^2/s.

D_G : The diffusion coefficient of G was measured as 6.7×10^{-7} cm^2/s in the brain [10] and 1.3×10^{-6} cm^2/s in collagen gel [11]. The diffusion coefficient in a growing tumor spheroid or aggregate is much smaller than the one in the medium, and so we take $D_G = 2.31 \times 10^{-7}$ cm^2/s.

chemo/hapto-taxis coefficients : χ_n, χ_n^1

In the presence of EGF, glioma cells travelled a distance 0.4-0.5cm in 150 hrs [12]; glioma cells in agar containing EGF travelled faster, covering a distance of 1.25cm in 150 hrs, while in plain agar they travelled only 0.75cm during the same period. In experiments with U87MGmEGFR spheroid growth, Deisboeck *et al.* [13] calculated the cell velocity to be in the range of $50-110$ $\mu m/24h$. Kim *et al.* [14] assumed that gradient of the glucose concentration was $3\times10^{-3}-10^{-4}$ g/cm^4 and a drift velocity $25-110$ $\mu m/24h$ of mobile cells to compute an intermediate value of $\chi_n = \frac{velocity}{gradient} = 2.76 \times 10^{-4}$ $cm^5/(g.s)$. In this paper, we assume that the chemotactic sensitivity is relatively small due to the fluctuating glucose level. We take $\chi_n=1.86 \times 10^{-7}$ $cm^5g^{-1}s^{-1}$.
For the haptotactic sensitivity, we take $\chi_n^1 = 4.17 \times 10^{-5}$ $cm^5/(g.s)$.

other parameters

In the next subsection we shall determine reference values n^*, ρ^*, P^*, G^* for n, ρ, P, G.

λ_{11} (tumor cell proliferation) : Doubling time were in the range from $27h$ (U87MG) to $60h$ (LN405) for human glioma cells [15]; this translates into proliferation rate of $(7.1-8)\times10^{-6}s^{-1}$. Measured value of proliferation rate were reported as $1/day$, or $8\times10^{-6}s^{-1}$, in typical experiments of Sander and Deisboeck [8]. Taking into consideration that large flux of glucose being supplied periodically in our system, we take $\lambda_{11}=1.112 \times 10^{-4}$ s^{-1}.

λ_{31} (MMP production) : It is difficult to measure the MMP production rate directly. The range of $(1.11-6.94)\times10^{-8}$ s^{-1} was estimated in [14] for sparse migrating cells. The MMP production rate,

written as $\lambda(n,\rho)$, was modeled by $\lambda_{31}n$ in [14] where $\lambda_{31} = 6.94 \times 10^{-8}\ s^{-1}$. Here, we model it as $\lambda_{31}n\rho$, because ρ is expected to oscillate quite significantly. In order to adjust to the order of magnitude of MMP production in [14], we take an estimated value, $\lambda_{31} = 6.95 \times 10^{-5}\ cm^3 g^{-1} s^{-1}$; see also [15].

λ_{32} (decay rate of MMP) : MMP is secreted by a tumor cell and is highly localized (fast decay) in the invading front of migrating tumor cells. We assume half-life of MMP to be very short (approximately 3.8 h) so that $\lambda_{32} = 5.0 \times 10^{-5}\ s^{-1}$.

λ_{41} (consumption rate parameters) : Nutrient consumption rate was measured as $\alpha = 1.6\ pg/cell/min$ in [16]. We compute λ_{41} from $\lambda_{41}G^\dagger = \alpha$ when $G^\dagger = 8.9\times10^{-4}\ g/cm^3$ is between high ($4.5\times10^{-3}\ g/cm^3$) and low ($3.0 \times 10^{-4}\ g/cm^3$). Hence, $\lambda_{41} = \frac{\alpha}{G^\dagger} = 0.3\ cm^3/(g.s)$.

Table 2 summarizes all the above parameter values.

Nondimensionalization

Tumor module

Table 3 lists reference values. We take $T = 1\ hour$ and $L = 1.0\ mm$, so that so that $D = \frac{L^2}{T} = 2.78 \times 10^{-6}\ cm^2/s$. We determine the reference values for n, ρ, P, G as follows:

n^* : We take n^*=$1 \times 10^{-3}\ g/cm^3$. This is based on Chicoine *et al.* [12] who plated human glioma cells with density 2.5-5$\times10^5$ $cells/ml$ in agar gel to investigate the cell migration in the presence of growth factors.

ρ^* : The main ECM component is collagen. Kaufman *et al.* (2005) investigated several patterns of different collagen I concentrations, and estimated ρ to be 0.5-2.0 mg/ml for glioma spheroids of diameter $\sim 200\mu m$. Stein *et al.* (2007) reported on experiments where U87 and U87ΔEGFR were implanted into collagen I of concentration of 2.6 mg/ml. We take ρ^*=$1.0 \times 10^{-3}\ g/cm^3$ as our reference value of ECM as in [14].

P^* : Recently, it was observed that PCK3145 has the ability to downregulate MMP-9 level for prostate cancer patients with high levels of MMP-9 $> 100\ \mu g/l$ [17]. We take $P^* = 1.0 \times 10^{-7}\ g/cm^3$ as in [14].

G^* : Sander and Deisboeck (2002) [8] used the characteristic concentration of glucose $2 \times 10^{-4}\ g/cm^3$, and took the boundary condition $6 \times 10^{-4}\ g/cm^3$ for glucose concentration far from the tumor (see also [13]). In [18], high ($4.5\ g/l = 4.5 \times 10^{-3}\ g/cm^3$) and low ($0.3\ g/l = 3.0 \times 10^{-4}\ g/cm^3$) glucose levels were introduced. We take this high value as a reference value so that dimensionless value of glucose ($G = 1$) corresponds to the high glucose level.

We nondimensionalize the variables and parameters in the partial differential equations (11)-(14) as follows:

$$\bar{t} = \frac{t}{T},\ \bar{x} = \frac{r}{L},\ \bar{n} = \frac{n}{n^*},\ \bar{n}_0 = \frac{n_0}{n^*},\ \bar{G} = \frac{G}{G^*},\ \bar{\rho} = \frac{\rho}{\rho_*},\ \bar{\rho_0} = \frac{\rho_0}{\rho_*},\ \bar{P} = \frac{P}{P^*},$$
$$\bar{D}_n = \frac{D_n}{D},\ \bar{D}_P = \frac{D_P}{D},\ \bar{D}_G = \frac{D_G}{D},\ \bar{\lambda}_{11} = T\lambda_{11},\ \ \bar{\lambda}_{21} = T\lambda_{21}P^*,$$
$$\bar{\lambda}_{22} = \lambda_{22}T,\ \bar{\lambda}_{31} = \frac{\lambda_{31}Tn^*\rho^*}{P^*},\ \bar{\lambda}_{32} = T\lambda_{32},\ \bar{\lambda}_{41} = T\lambda_{41}n^*,\ \bar{\lambda}_{42} = \frac{T\lambda_{42}}{G^*},$$
$$\bar{\chi}_n = \frac{\chi_n G^* T}{L^2},\ \bar{\chi}^1_n = \frac{\chi^1_n \rho^* T}{L^2}.$$

We choose λ_G and λ_ρ such that $\bar{\lambda}_G = \bar{\lambda}_\rho = 1$. If we drop the bar ("-") in the new variables and parameters, then the differential equations remain unchanged.

Internal dynamics module

In order to mathematically model the dynamical network shown in Figure 3 the dimensional version of the dynamical system for the internal dynamics was derived as follows:

$$\frac{dm}{dt} = g + \frac{\Lambda_1 \Lambda_2^2}{\Lambda_2^2 + \Lambda_5 a^2} - \mu_1 m,$$
$$\frac{da}{dt} = s + \frac{\Lambda_3 \Lambda_4^2}{\Lambda_4^2 + \Lambda_6 m^2} - \mu_2 a,$$

where $m(t)$ and $a(t)$ are concentrations of miR-451 and the AMPK complex at time t, s is source of the complex, g is the glucose level within the experimental system, Λ_1 and Λ_3 are the autocatalytic enhancement parameters for miR-451 and the complex respectively, Λ_2 and Λ_5 are the Hill-type inhibition saturation parameters from the counter part of miR-451 and the complex respectively, Λ_5 is the inhibition strength of miR-451 by the complex, Λ_6 is the inhibition strength of the complex by miR-451, μ_1 and μ_2 are the decay rates of miR-451 and the complex respectively.

The following dimensionalization was performed to get the dimensionless key control equations in the main section

$$T = \mu_1 t,\ M = \frac{m}{m^*},\ A = \frac{a}{a^*},\ G = \frac{g}{\mu_1 m^*},\ S = \frac{s}{\mu_2 a^*},\ k_1 = \frac{\Lambda_1}{\mu_1 m^*},$$
$$k_2 = \Lambda_2,\ k_3 = \frac{\Lambda_3}{\mu_2 a^*},\ k_4 = \Lambda_4,\ \alpha = \Lambda_5 (a^*)^2,\ \beta = \Lambda_6 (m^*)^2,\ \epsilon = \frac{\mu_1}{\mu_2}.$$

miRs are typically more stable than their targets [19, 20] and the parameter ϵ is small [21]. Typical half-life of AMPK is measured to be 6 h [22] ($\mu_2 \sim 0.12 h^{-1}$) while the half-life of a miRNA is much larger 101-225 h in a recent study [23]. We take a slightly larger half-life of miR-451, 290 h, leading to $\mu_1 = 0.0024\ h^{-1}$ and $\epsilon = \frac{\mu_1}{\mu_2} = 0.02$. miRNA concentrations in an animal cell (assuming 1000-25,000 μm^3 volume) were estimated to be 80 $pM - 2.2\ \mu M$ [24] and we take our reference value $m^* = 1.0\ \mu M$. Based on the high (4.5 g/l) and low (0.3 g/l) glucose level in [18] and m^*, we estimate glucose supply rate through several pathways $g = (2.4 \times 10^{-5} - 2.4 \times 10^{-3})\ \mu M/h$ resulting in a range of dimensionless glucose input levels $G = \frac{g}{\mu_1 m^*} = 0.01 - 1.0$. AMPK concentration was measured as 35-150 nM in rat liver [25] and we take $a^* = 100 nM$. We take the signal source of the AMPK complex, $s = 2.4\ nM/h$ leading to $S = \frac{s}{\mu_2 a^*} = 0.2$. The autocatalytic rate (Λ_1) of miR-451 is assumed to be 4-fold larger than its negative contribution ($\mu_1 m^*$) from its decay in the absence of inhibition pathway from the AMPK module, $k_1 = \frac{\Lambda_1}{\mu_1 m^*} = 4.0$ (Similarly for its counterpart, the AMPK complex, we take $k_3 = \frac{\Lambda_3}{\mu_2 a^*} = 4.0$). The Hill-type dimensionless parameters Λ_2, Λ_4 (and their corresponding k_2, k_4 without change) are fixed to be equal to 1. Finally, the inhibition strength ($\alpha = 1.6$) of miR-451 by the AMPK complex was assumed to be a bit stronger than the inhibition strength ($\beta = 1.0$) of the AMPK complex by miR-451.

Table 1 summarizes all the above parameter values for the internal dynamics model (1)-(2).

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