Cusp bifurcation in a metastatic regulatory network

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ABSTRACT

Understanding the potential for cancers to metastasize is still relatively unknown. While many predictive methods may use deep learning or stochastic processes, we highlight a long standing mathematical concept that may be useful for modeling metastatic breast cancer systems. Ordinary differential equations (ODEs) can model cell state transitions by considering the pertinent environmental variables as well as the paths systems take over time. Bifurcation theory is a branch of dynamical systems which studies changes in the behavior of an ODE system while one or more parameters are varied. Many studies have applied concepts in one-parameter bifurcation theory to model biological network dynamics, and cell division. However, studies of two-parameter bifurcations are much more rare. Two-parameter bifurcations have not been studied in metastatic systems. Here we show how a specific two-parameter bifurcation phenomenon called a cusp bifurcation separates two qualitatively different metastatic cell state transitions modalities and propose a new perspective on defining such transitions based on mathematical theory. We hope the observations and verification methods detailed here may help in the understanding of metastatic potential from a basic biological perspective and in clinical settings.

1. Introduction

Different cell states can emerge during disease progression, such as cancer metastasis. Regarding metastatic cancer, much attention has been devoted to two cellular states: epithelial (E) and mesenchymal (M), each recognizable by the levels of specific proteins, which correspond to the steady states of multistable gene regulatory networks (Kalluri and Weinberg, 2009; Hanahan and Weinberg, 2000; Addison et al., 2021; Thiery and Sleeman, 2006; Mladinich et al., 2016). Normal epithelial (E) cells are not motile and can grow (divide) in response to growth signals, as opposed to mesenchymal (M) cells that do not form epithelial layers and are motile, but less likely to divide (Kohrman and Matus, 2017). The assumption of a binary choice between cell division and movement is the "go-or-grow hypothesis" (Hoek et al., 2008). Since metastasis requires departure from a primary site and growth at a different site, both the epithelial-mesenchymal transition (EMT), and mesenchymal-epithelial transition (MET) seem to be required (Brabletz, 2012). Thus, EMT alone is not always sufficient for metastasis (Fischer et al., 2015) if a binary classification into E and M cell types is assumed, since MET is also required for growth in the new location. Moreover, EMT might not be necessary either, according to recent work (Fischer et al., 2015;

Zheng et al., 2015) showing that most metastatic cells do not undergo full EMT, and EMT inhibition does not reduce metastasis. Furthermore, metastasis relies on detached cells invading their tissue neighborhood and accessing the bloodstream, which occurs on top of EMT, under the control of different genes called pro- and antimetastatic regulators, such as BACH1 and RKIP (Lee et al., 2014). We show how to pinpoint the threshold when these regulators cause a qualitative shift in the transition between the two states, and describe a new view that may be crucial to understanding whether and how metastasis will occur.

Many recent studies indicate that EMT and MET are more complex than binary processes, i.e., they are transitions between more than two distinct, well-defined cellular states (Zhang et al., 2014; Jolly et al., 2017; Lu et al., 2013). One or more intermediate, "hybrid" or "partial" EMT cell states with mixed E/M properties have been described (Aiello et al., 2018; Selvaggio et al., 2020; Pastushenko et al., 2018). Recent computational work that varied the number of hidden intermediate states, aiming to improve fits to experimental data (Goetz et al., 2020) found that intermediate states can accelerate EMT. It seems possible that only these intermediate EMT states, instead of full EMT, are necessary and sufficient for metastasis (Simeonov et al., 2021). In general, the number of such intermediate states is unknown, raising the

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question: Can the number of intermediate states go to infinity, allowing continuous transitions? And what defines the boundary between such continuous transitions versus the widely-studied discrete EMT and MET transitions, with a finite number of distinguishable steady states? Finally, how can these theoretical questions help us to understand the biology of metastasis?

The use of equilibrium states of differential equations as a model for biological or chemical systems including metastasis-regulatory networks, has a long history which we do not try to survey here, except for a few instances. (Waddington, 1940; Delbrück, 1949; Thom, 1969, 1975; Kauffman, 1993; Cherry and Adler, 2000; Gardner et al., 2000; Elowitz and Leibler, 2000) The study of bistable systems has played an important role, especially noteworthy are the works on the toggle switch (Cherry and Adler, 2000; Gardner et al., 2000) and on direct and indirect fully positive feedback (Becskei et al., 2001; Ferrell, 2002). (A deeper example of these models and how they relate to cusp bifurcations can be found in the Supplementary Materials)

Mathematical studies of genetic regulatory networks have usually relied on solving the corresponding differential equations numerically or with the use of topological or fixed point techniques and theorems from one-parameter bifurcation theory to prove the existence of solutions with particular properties. One example of the use of topological methods describing critical points and associated Boolean networks is by Glass (Glass, 1975). An example of the extensive use of numerics is Lee et al. (2014). By contrast, the use of two-parameter bifurcation theory rarely if ever enters into the biological theory, even in papers with the word bifurcation in the title as in Spencer et al. (2013) or in the text as in Rajapakse and Smale (2017). The cusp bifurcation is a concept from two-parameter bifurcation theory, found by solving a system of equations in the state and parameter variables. Here we present methods for finding cusp points and give a self-contained elementary derivation of a set of equations and their solution for finding cusp points. We provide below a simple computational framework to find accurate solutions to such systems. We show how this provides a broader view on the emergence of bistability in biological systems, by dividing the two-dimensional parameter space into regions with distinct transition types: continuous and discontinuous transitions. This should improve the conceptual understanding of cell state transitions in metastatic gene networks and other biological systems.

2. The cusp point separates two kinds of cell state transitions

Cell states or "cell types" (Rajapakse and Smale, 2017) have been modeled as stable steady states, or equilibria, of ordinary differential equations

$$\dot{x} = V(x, \alpha)$$

such that $x \in \mathbb{R}^n$ is a concentration vector representing the cell's molecular composition and $\alpha \in \mathbb{R}^j$ is a vector of j parameters, representing internal and external characteristics, including reaction rates. Steady states correspond to the values of x_α which satisfy $V(x_\alpha, \alpha) = 0$. If the eigenvalues of the derivative of $V(x_\alpha, \alpha)$, denoted $D_x V(x_\alpha, \alpha)$, all have negative real part then nearby solutions all tend to x_α as time increases. It is possible that $V(x, \alpha)$ may have a unique stable state, multiple stable states or even more complicated dynamical behavior. Each stable equilibrium $x_{\alpha,i}$ corresponds to a cell type. If the parameter α depends on some external factor f_{ext} because of sensory, genetic, epigenetic, spatial or other effects, then the number and value of stable states $x_{\alpha(f_{ext})}$ may depend on $\alpha(f_{ext})$, which describes the dynamical behavior of cells transitioning from one type to another dependent on f_{ext}, which could be the time variable. We are interested in studying the transitions between pro-metastatic and anti-metastatic mono-stable states of cells, analogous to EMT and MET, using mathematical models derived from bifurcation theory. In the model $\dot{x} = V(x, \alpha)$, the variables $x(t)$ that represent proteins involved in metastatic cell transitions are time-dependent. The parameters $\alpha(f_{ext})$ depend on an external factor,

which could be a chemical concentration, cell size, or time. As long as $\alpha(f_{ext})$ is a continuous function of f_{ext}, the theory we present is valid. If the factor $f_{ext} = t$ is time, as we assume in the following for simplicity, the adjustment time scale of the steady state would be faster than the time scale of $\alpha(t)$, as usually assumed in bifurcation theory. We will mainly focus on cell types in metastatic breast cancer, but such analyses may also be generalized to other metastatic cancers, biological networks orchestrating events such as cell division, Spencer et al. (2013) or synthetic gene circuits (Rajapakse and Smale, 2017).

Smale and Rajapakse refer to cell states as "cell types" in Rajapakse and Smale (2017) where they identify conditions in biological networks for which a pitchfork bifurcation in 2 and 3 variable systems with one parameter exist. Yet, the pitchfork is a one-parameter bifurcation that may not be stable. There is a stable two parameter bifurcation called the cusp bifurcation, which includes a pitchfork as a one dimensional sub-bifurcation. The applicability of the cusp bifurcation or two-parameter bifurcations to metastatic transitions has not been widely investigated. Here we use known methods (Pujals et al., 2020) to verify that a pitchfork exists in the metastasis model by Lee et al. (2014) (See sections 2.2 and 2.3). Moreover it can be shown that all the examples of pitchfork bifurcations proven by Smale and Rajapakse in Rajapakse and Smale (2017) concerning "Repressillator" and "Toggle" synthetic gene circuits are actually one dimensional sub-bifurcations of cusp bifurcations (See Supplementary Materials). We plot the cusp curve in the metastatic breast cancer model, which is the projection of the fold onto the parameter space and observe that it divides the parameter space into two regions separated by the curve and cusp point, which correspond to biological transitions of two types. These transition types differ in the two types of paths taken by the curve $\alpha(t)$. The first goes around the cusp point, and has no bistable points, whereas the second crosses the bistable region.. The first we call a continuous transition, as it may capture the biological phenomenon of not just one, but any number of "hybrid" or "partial" cell types, differently than in previous studies. Specifically, instead of multiple equilibria, we find that there is always only one stable equilibrium in the dynamical system, corresponding to a continuum of partial cell types, which may be sufficient for metastasis. The second transition is a discontinuous transition between binary cell types, or anti-metastatic and pro-metastatic cells, which happens when a stable equilibrium bifurcates and two stable equilibria and one unstable equilibrium (saddle) appear. In the continuous regime, noise or external fluctuations might easily confer partially metastatic phenotypes to cells, as opposed to the discrete regime, where cells are more robust to such perturbations.

Overall, we show a novel application of bifurcation theory in biology, propose a shift from continuous (non-binary) to discrete (binary) transitions at the cusp point, and discuss further applications of these concepts in metastasis and other biological phenomena. We have also provided a tutorial in bifurcation theory and any relevant code in the Supplementary Materials, which others may find useful for their own research.

3. Cusp bifurcation for metastatic cell state transitions

3.1. Analytical methods for finding the cusp point: The metastatic cell transition ODE model

First, we derive mathematical conditions that can identify a cusp bifurcation using the model from the paper "Network of mutually repressive metastasis regulators can promote cell heterogeneity and metastatic transition" (Lee et al., 2014). Here, Lee et al. considered three differential equations with two parameters $V(R, L, B, \rho, k)$ where R, L, B are real positive variables and ρ, k are real positive parameters (Fig. 1(a)). R, L, B represent the proteins and RNAs RKIP, let-7 and BACH1 which interact in the cell (Fig. 1(b)) and are highly relevant for determining breast cancer metastasis. The parameters ρ and k describe the instability of RKIP and insensitivity of BACH1 to self-regulation, respectively. The equations are
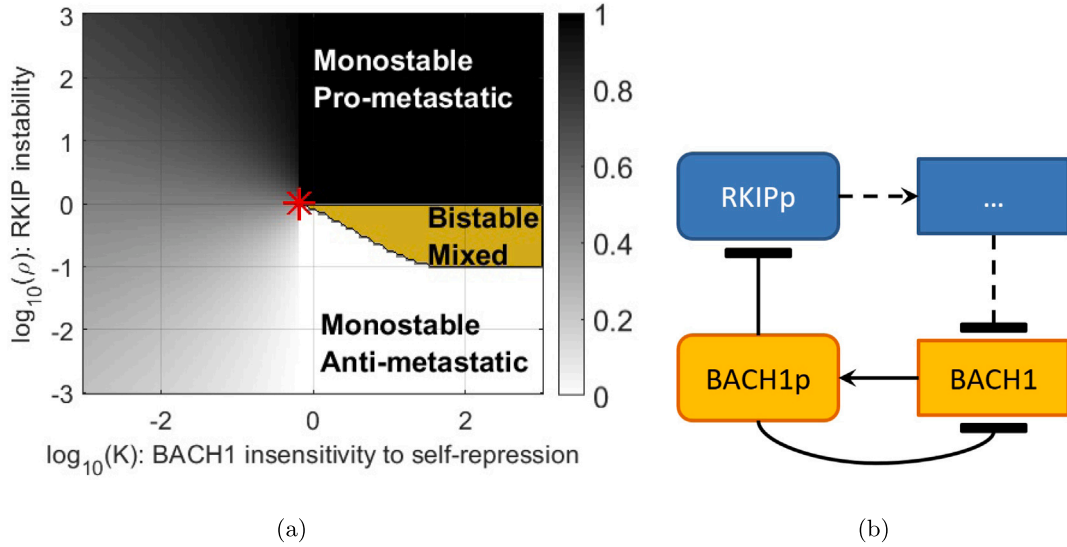

(a)

(b)

Fig. 1. (a) Map of dynamics from Lee et al. (2014). Grayscale shading indicates monostability. Darker shading corresponds to higher BACH1 levels. Yellow color indicates bistability. The red star indicates the expected cusp point where $(\rho, K) = (1.0024, 0.6595)$. (b) Gene network diagram of BACH1 and RKIP regulatory interactions. BACH1 is BACH1 gene, BACH1p is BACH1 protein; RKIPp is RKIP protein. The dots represent intermediary regulators between RKIP and BACH1.

$$\frac{dR}{dt} = \frac{1}{1+B} - \rho R$$
$$\frac{dL}{dt} = \frac{aR^r}{m^r + R^r} - L - cLB \qquad\qquad \equiv V(R, L, B, \rho, k)$$
$$\frac{dB}{dt} = s + \frac{(S-s)k^b}{k^b + B^b} - B - cLB$$

The constants are set to $s = .02, S = 20, c = 200, m = 2, b = 3, r = 5, p = 10, a = 1000$. Let $\vec{x} = (R, L, B)$ so for convenience we may write $V(\vec{x}, \rho, k)$ and take derivatives with respect to $\vec{x}$.

Observable cell states are equilibria of the system $V(\vec{x}, \rho, k)$. For ρ, k fixed, equilibria are points $\vec{x}$ such that $V(\vec{x}, \rho, k) = 0$. The equilibrium is stable if the real parts of the eigenvalues of $D_x V(\vec{x}, \rho, k)$ are negative. All solutions of the ODE which start near a stable equilibrium tend to the equilibrium as time increases. An equilibrium may lose its stability and a bifurcation may occur as we vary (ρ, k) if one of the eigenvalues tends to have zero real part or more specifically, if the eigenvalue becomes zero. In other words, to find bifurcations, we are looking for the solutions of the determinant $Det[D_x V(\vec{x}, \rho, K)] = 0$.

Via an intricate analysis of the ODE, Lee et al. divide a region in the (ρ, k) parameter plane into three sub-regions (Fig. 1(a)). One region with a single stable equilibrium corresponding to an anti-metastatic state of the cell, one with a single stable equilibrium corresponding to a pro-metastatic state of the cell and one with three equilibria, two of which are stable. This suggests that as ρ, k vary the state of the cell may start in one monostable region and pass through a bistable region to the other monostable region. Thus the boundary separating the bistable and mono-stable regions is a curve of interest. It is defined by the solution of

$$V(\vec{x}, \rho, k) = 0$$
$$Det[D_x V(\vec{x}, \rho, k)] = 0$$

There are now 4 equations in 5 unknowns to solve. We assume that the rank of the derivative of this system is 4 when the equations are satisfied. By the implicit function theorem we may graph a curve for an underdetermined system (Pugh, 2015) to (locally) locate the set of solutions. The curve projected onto the (ρ, k)-plane is smooth and locally separates the regions of the plane. Under certain conditions this curve may meet at a cusp point (See Supplementary Materials) . The shape of the bistable region is such that we suspected that there is a cusp point, which we do in fact find to be close to the red star in Fig. 1(a).

A cusp point is the (ρ, k) parameter coordinates of a non-degenerate solution of the following five by five system of equations.

$$V(\vec{x}, \rho, k) = 0$$
$$Det[D_x V(\vec{x}, \rho, k)] = 0 \qquad\qquad (1)$$
$$\nabla_x Det[D_x V(\vec{x}, \rho, k)] \bullet (\vec{v}) = 0$$

Here ∇_x denotes the gradient in terms of $\vec{x}$ which we take of the determinant of the derivative. We then calculate the dot product with $(\vec{v})$ which is the first column of the adjugate matrix of $D_x V(\vec{x}, \rho, k)$. We assume $(\vec{v})$ is not zero. The rank of $D_x V(\vec{x}, \rho, k)$ is two where the equations are satisfied because $D_x V(\vec{x}, \rho, k)$ is a 3 by 3 matrix with an eigenvalue equal to zero. Since the map has maximal rank, it is stable even if the parameters vary slightly. The solution to Eq. (1) define the values of proteins R, L, B and the parameter values ρ, k required at the cusp point.

In order to observe metastasis, cells must traverse from the bottom to the top and back in Fig. 1(a). To the left of the cusp, which is the gray area of Fig. 1(a), we have cells that can transition continuously from one state to another (Fig. 2(a)). Noise or environmental perturbations can easily cause such changes. To the right of the cusp, however, cells must cross the bistable region in yellow in the upward direction and then again in the downward direction, where the cell plots are discontinuous (Fig. 2(b)), implying higher robustness to noise or environmental perturbations. We explain this further in the Discussion section below. Mathematically, these paths can be observed by finding solutions of the system where either k or ρ are fixed. In the first case, k is fixed and must be less than its cusp point value, and in the second k must become greater than the cusp point value. In Fig. 2 we draw sketches of what we expect these solutions to look like and how they relate to metastasis.

3.2. Numerical approach for finding the cusp point and model verification

We built a Newton's Method algorithm in MATLAB for an underdetermined system of equations to validate the ρ, k solution values we identified from Lee et al. We used MATCONT (Dhooge et al., 2008), a continuation toolbox for ODEs, to isolate a more accurate set of values for the cusp point on the (ρ, k)-plane and plot the projection of the cusp curve. We also built codes in MATLAB to visualize the various behaviors of the system which can be found in the Supplementary Materials.
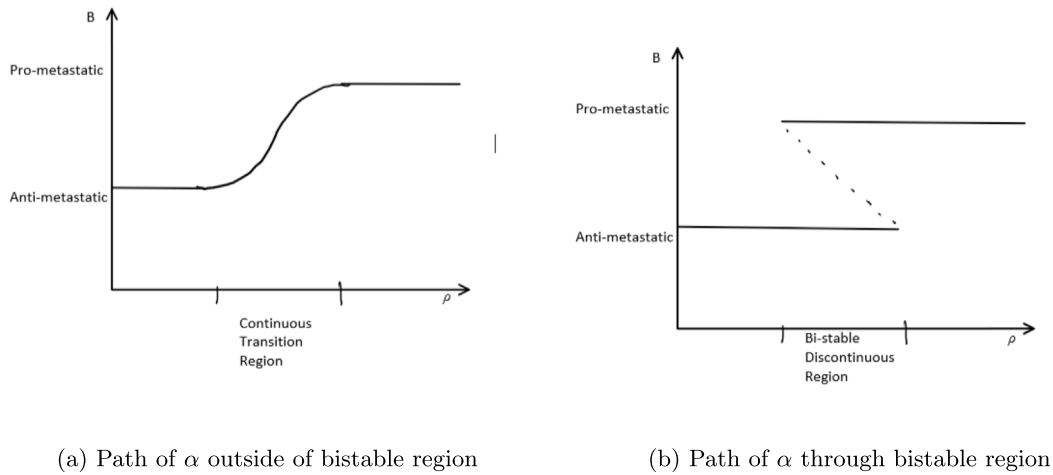
(a) Path of α outside of bistable region

(b) Path of α through bistable region

Fig. 2. Two different modes of cell state transitions between monostable regions. Image (a) demonstrates a continuous transition cell state path and (b) demonstrates a bistable discontinuous transition cell state path.
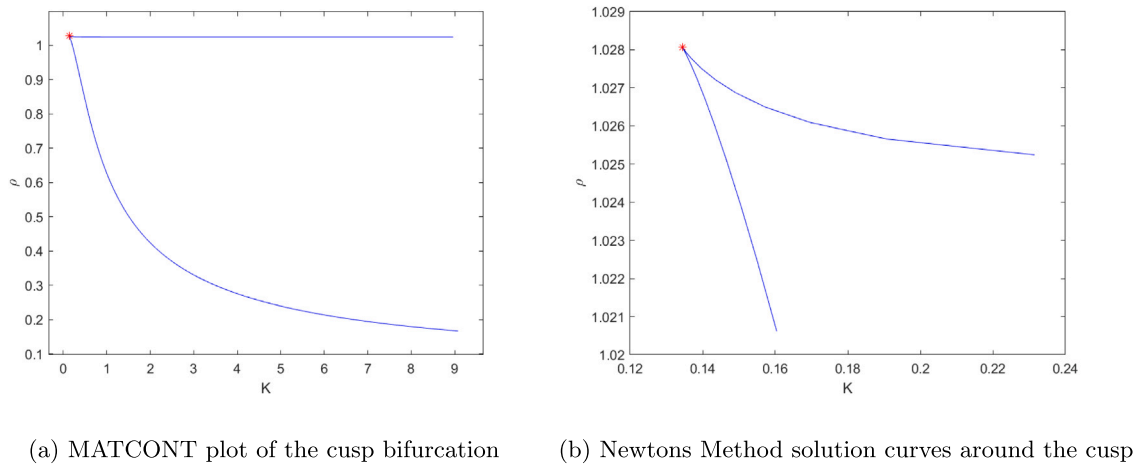

(a) MATCONT plot of the cusp bifurcation

(b) Newtons Method solution curves around the cusp

Fig. 3. The cusp point plot in (a) is the output from MATCONT when running the Limit Cycle differential equation solver for a Cusp Point. In plot (b) we used our Newton's method algorithm to verify numerically the same plane division using the equations from Lee et al. The star marks the MATCONT generated values of $(\rho, k) = (1.0281, 0.1343)$.

To validate this is in fact a cusp point, we solved the system of equations in MATCONT to find that the cusp is located at $(\vec{x}, \rho, k) = (0.9321, 2.2184, 0.0435, 1.0281, 0.1343)$. Note that the values from MATCONT return $\rho, k = (1.0281, 0.1343)$ whereas the previous values in Fig. 1(a) were $(1.0024, 0.6595)$. When plotting ρ, k we found a similar separation of the regions defined by Lee et al. Fig. 3.

Next, we numerically verified that the five by five system has an invertible derivative at the solution. At a cusp point the tangent to the cusp exhibits a pitchfork bifurcation. Pujals et al. (2020)

Fig. 4 is a plot of the pitchfork bifurcation around the cusp point $(\rho, k) = (1.0281, 0.1343)$ where the tangent vector is $(-0.1542, 1)$. For values of T moving in the positive direction of the tangent, B consistently has 3 solutions, two stable equilibria which are the upper and lower branches of the pitchfork and one unstable equilibria in between. In the negative direction of T we find only 1 solution.

We explore how varying values of ρ affects B in Fig. 5. First we note that near the cusp point for different values of $k > .13$ we see a similar curve. If we follow a solution from the lower monostable region it disappears as ρ increases, at 1.024. This is the first limit point (LP) or equilibrium in the graph. Then B increases towards the value corresponding to the other stable equilibrium which is the upper limit point. The curve between the two limit points represents the bistable region which we showed in Fig. 2(b). The bistable region in Fig. 1(a) corresponds to the unstable equilibrium and the space between the two
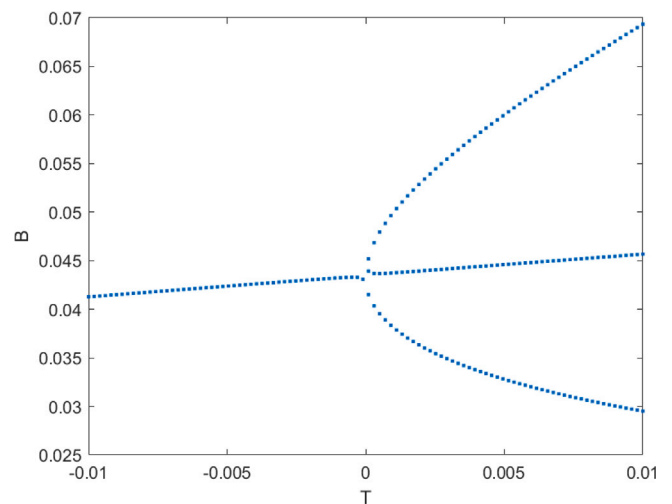

Fig. 4. Pitchfork Bifurcation plot. The solution of B is derived around the new values of $(\rho, k) = (1.028071, 0.134353)$ using the Matlab function vpasolve().

limit points. At the second limit point B crosses from the bistable to the monostable region. As ρ increases, B stays in the upper branch of
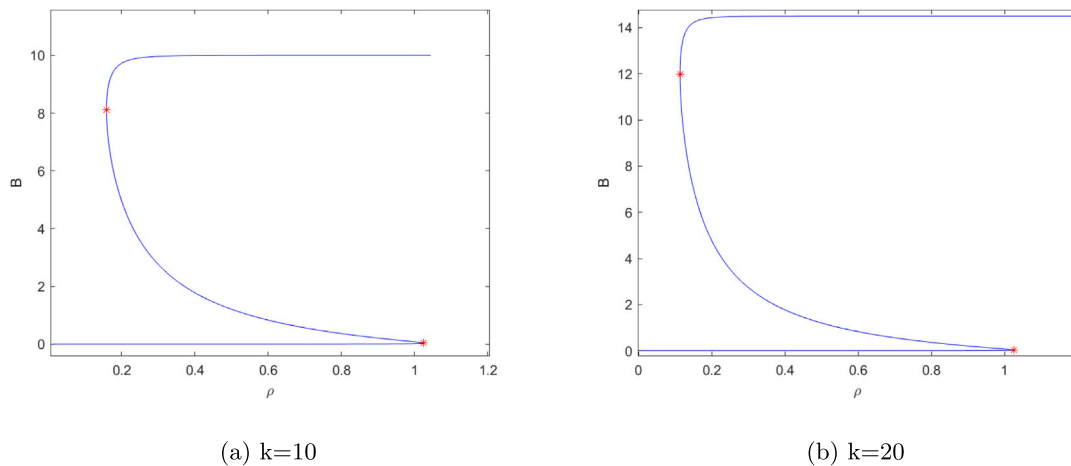
(a) k=10

(b) k=20

Fig. 5. (a) is a solution curve from MATCONT's Equilibrium curve solver where $k = 10$ was fixed and ρ varied. (b) is a solution curve from MATCONT's Equilibrium curve solver where $k = 20$ was fixed and ρ varied. In both plots the red stars are limit points, demonstrating discontinuous solutions to the system.

the curve. As ρ is approaching zero B tends towards the upper limit point. The limit points are where ρ crosses the cusp line for fixed k, thus demonstrating hysteresis. We may interpret this as having a concentration of antimetastatic cells for values of $\rho = \{0, 1.024\}$ and a concentration of prometastatic cells from $\rho = \{.761, \infty\}$ which "mix" in the bistable region.

In Fig. 6, if $k = .13$ or less than the value required at the cusp, the solution curve is S shaped and B is increasing continuously as ρ increases. As we continuously increase ρ we pass from the anti-metastatic to pro-metastatic cell states as in Fig. 2(b). In this scenario, if points on the solution curve correspond to various cell types, we could posit that we transition to many intermediate cell states as we go from one mono-stable state to another. This differs from the case when $k > .13$ where we see two distinct sets of cell types.

Finally, in Fig. 7 we plot the surface B. If we intersect the solution plane at a fixed value of k the solution curves are similar to Fig. 5 depending on the choice of k. If we intersect the plane at the cusp point where the surface folds we have a solution curve which looks like a pitchfork, as demonstrated in Fig. 4. Furthermore, if we project the fold lines of the surface onto the ρ, k plane we end up with a curve and cusp point as seen in Fig. 6.

4. Discussion

Many attempts to predict metastasis have been made using a variety of clinical methods and computational techniques (Jin et al., 2020; Jiang et al., 2021; Zaritsky et al., 2021). One great challenge is understanding how the environment of early stages of cancer may determine metastasis later on. None of the earlier studies of one-parameter EMT regulatory network dynamics incorporate the information we have found regarding cusp bifurcations in metastatic systems, which we model differently from the earlier EMT studies, by using two-parameter bifurcation theory to analyze a different gene network directly involved in metastasis. Since the theory of two-parameter bifurcations and cusp bifurcation analysis is generally applicable, we believe these findings may complement one-parameter bifurcation studies on EMT and enhance existing attempts to develop predictive methods. In our study we have identified a cusp bifurcation in the model provided by Lee et al. which suggests to us that an interesting behavior, similarly important as hysteresis is for one-parameter saddle–node bifurcations, may occur in metastasis more generally, where the path of the system will determine final metastatic states. It may even be possible to find this behavior in other cancers or cancer-related phenomena governed by bistable or multistable regulatory networks. Below we describe one possible interpretation of how the cusp may determine certain distinct system pathways towards metastasis.
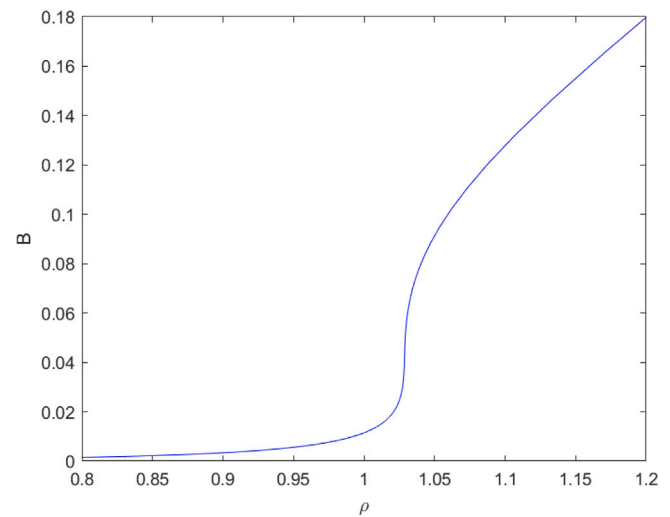

Fig. 6. A continuous solution curve plot from MATCONT's Equilibrium curve differential equations solver. This image is meant to demonstrate how varying ρ for a fixed value of $k < .13$ will yield a continuous uninterrupted curve without any cusp or limit points.

In Fig. 8 we have superimposed potential paths of $\alpha(t)$ in the space of parameters ρ and k from Fig. 1(a).

The time-evolution of the parameters $\alpha(t) = (\rho, k)$ shows possible transitions between stable cell types. Here, the green line indicates how values of $\alpha(t)$ begin in the monostable anti-metastatic region (A), traverses an ambiguous monostable region and arrives into the monostable pro-metastatic region (P). These transitions we will call APT, or PAT depending on the direction. The unique stable state of the differential equations $x(t)$ evolves together with $\alpha(t)$ but the possible cellular type ultimately transitions between pro-metastatic and anti-metastatic states. Therefore this image represents a continuous transition depending on which way the path is traversed. It is important to note that both transitions traverse the ambiguous region to the left of the bistable region. Cells in this ambiguous region are neither pro- nor anti-metastatic. They are in an intermediary, biologically ambiguous cell state where the pro-and antimetastatic states become indistinguishable. Such ambiguous parameter regions must also appear in all other analyses describing bistable or multistable systems, but they have not been mathematically characterized from the perspective of metastasis. Cells in this region, or even in the bistable region near the
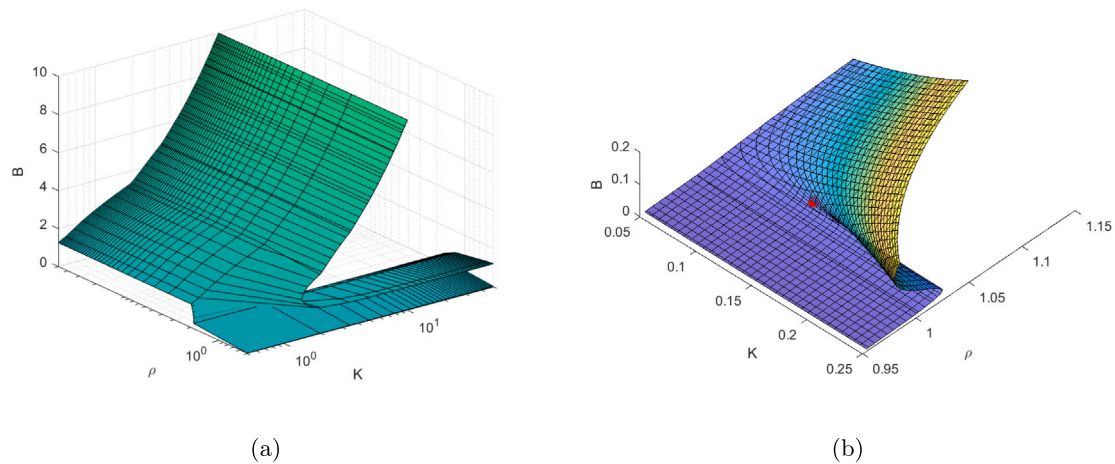
Fig. 7. (a) Cusp fold plot of ρ and k using the Matlab function fimplicit3(). At fixed values of k we see ρ varies continuously, transitioning from regions above and below the fold. At the cusp point the surface intersection along fixed k is a pitchfork. The projection of the surface around the cusp onto the ρ, k would yield the cusp point plots above. (b) Cusp fold image zoomed in near the cusp point in red.
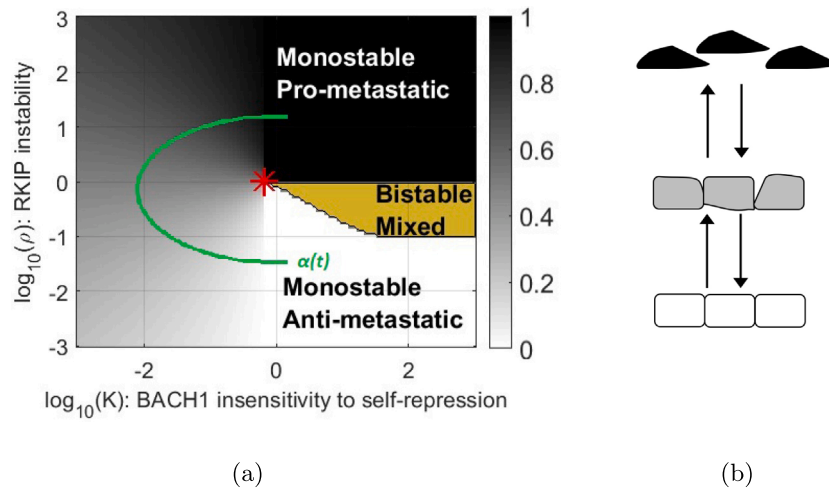

Fig. 8. (a) Time-evolution exclusively through monostable regions. Every point on the path is monostable. (b) Image of monostable cells. Pro-metastatic cells in black, Anti-metastatic cells in white and intermediate monostable cells in continuous transition region in gray.

cusp can easily assume such ambiguous states and become metastasis-prone due to environmental effects or noise. Understanding where this possibility arises requires finding the cusp point, which underlines the biological importance of our findings. Analogies may exist with the physics of phase transitions, e.g., liquid and gas phases becoming indistinguishable, enabling smooth, continuous transitions between phases beyond the critical point of water.

In Fig. 9 the path of $\alpha(t)$ crosses the boundary between the monostable and bistable regions. Similar transitions have been extensively described in the EMT/MET literature. At first we consider the path as it goes up transitioning from anti-metastatic to pro-metastatic (APT). As the path crosses the boundary at t_0, the edge of the bistable mixed region, the stable state $x(t)$ continues on while a new equilibrium point is created which we denote $x'(t_0)$. Over time the point $x'(t)$ splits into a new stable point $x'_1(t)$ and a saddle $x'_2(t)$. In the bistable region there are three equilibria $x(t)$, $x'_1(t)$ and $x'_2(t)$. If we cross the higher boundary of the bistable region a stable point and a saddle collide and annihilate each other that is, $x(t)$ and $x'_2(t)$ collide.

This would be a discontinuous transition in the direction A to P, since at every point along the path where the A and P states co-exist, they correspond to separate sets of variables x_A and x_P, which are clearly distinguishable. We illustrate this in 1-dimension (see Fig. 10). There is no ambiguity at any point about an individual cell being in one

state or another. But now as we run this path backwards to produce a PAT we see that the state of the cell corresponds to $x'_1(t)$ as $x(t)$ tends to $x'_1(t)$ in the bistable region. Thus traversing the path in one direction and then the other exhibits hysteresis. Hysteresis is another hallmark of discontinuity. Unlike continuous transitions, discontinuous EMT/MET has been extensively investigated for many regulatory networks (See also Fig. 2).

Now if we imagine that metastasis will require an APT transition followed by a PAT transition we see that the PAT transition may require that the parameter cross the whole bistable region in reverse. The region near or left of the star in Fig. 1(a) would seem to provide the most fertile region for such a transition in both directions. This applies to both continuous and discrete transitions, to the left and right of the red star, which indicates the cusp point. Two questions are immediate:

1. What determines the path $\alpha(t)$ of continuous or discontinuous transition that the cell will trace out in the parameter space?

2. Will the paths of the cells stay away from or pass close to the cusp point? What is the biological significance of this alternative? Will paths that pass close to the cusp be more likely to undergo an APT transition, followed by an PAT transition, and thus be more likely to establish distant tumors?

We posed these questions in connection with metastatic cell state transitions above. We also proposed the possibility of seeing the same
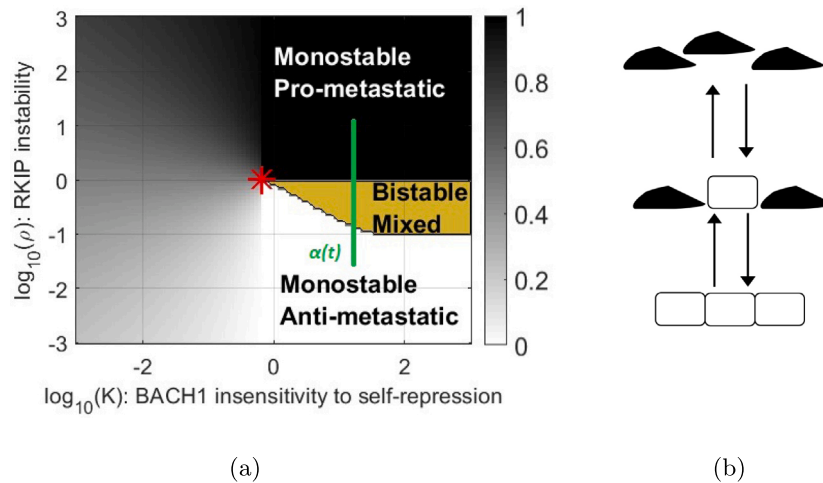
Fig. 9. (a) Transition between monostable regions traversing bistable mixed region. (b) Image of Discontinuous transition. Monostable Pro-metastatic cells in black, Monostable Anti-metastatic cells in white and cells in mixed Bistable region in both white and black.
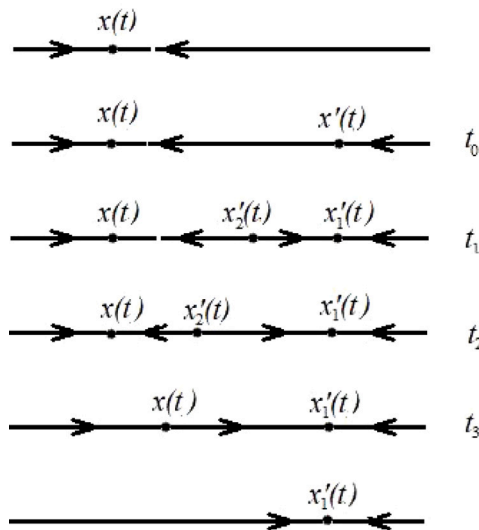

Fig. 10. 1-dimensional time phases of stable states.

behavior in 2 or 3-gene circuits and in cell division dynamics that mimic a "Toggle" network (See Supplementary Materials section 1.1.2, where an elementary model for the transition to cell cycle or differentiation is given). A general and in depth perspective on the mathematics behind finding a cusp bifurcation is introduced in the Supplementary Materials which we hope could be applied to other biological networks. It is possible that many more biological systems exhibit cusp and pitchfork bifurcations where a simple one-parameter binary transition may not sufficiently describe the biological phenomenon and we think that it is possible to show mathematically that these systems can undergo two-parameter bifurcations. If this is the case then we believe it is important to revisit existing studies of gene networks and apply these findings from two-parameter bifurcation theory to system models and predictive techniques.

We note that there is another approach to the use of bifurcation theory in biology. This is the Catastrophe theory of René Thom (Thom, 1975). The developed theory concerns the zeros of gradient vector fields and their bifurcations. There is a cusp bifurcation which is very much the same in its geometric features. However, one has to be a little careful here since the bifurcation theory of differential equations and gradient differential equations have some subtle differences. For

example the general cusp bifurcation has the possibility of exhibiting a periodic solution which the gradient system does not. The survey paper by Rand et al. (2021) has recent updates to this theory. Given a differential equation satisfying certain properties, there is a gradient system which shares the asymptotic behavior of the original system. Now Rand adds the unstable manifold geometry behavior to the analysis. A drawback of this theory may be that the gradient system is not immediately at hand, whereas we work with the equations directly.

Further work as it relates to metastatic breast cancer would be to incorporate these models to existing predictive processes that assess metastatic potential. Furthermore, it would be valuable to validate that similar cusp behaviors are found across other metastatic cancers more generally. Further interesting mathematical studies would be to solve the equations in Lee et al. and other existing metastasis models, symbolically to make sure we have all possible solutions. It would also be valuable to rigorously prove that the rank of the derivative is always 4 in such systems to validate our hypothesis. A forthcoming analysis of these initial results could be made to generalize the observation of cusp bifurcations in other bistable or multistable biological networks and extend two-parameter bifurcation theory in a variety of biological phenomenon.

CRediT authorship contribution statement

Brenda Delamonica: Conceptualization, Methodology, Software, Writing. **Gábor Balázsi:** Conceptualization, Supervision, Writing. **Michael Shub:** Conceptualization, Methodology, Supervision, Writing.

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.

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Appendix A. Supplementary materials

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.jtbi.2023.111630. (Contains a tutorial on bifurcation theory, cusp points identified in other biological systems, and relevant code.)

References

Addison, Joseph B., et al., 2021. Functional hierarchy and cooperation of EMT master transcription factors in breast cancer metastasis. Mol. Cancer Res. : MCR 19 (5), 784–798.

Aiello, Nicole M., et al., 2018. EMT subtype influences epithelial plasticity and mode of cell migration. Dev. cell 45 (6), 681–695.e4.

Becskei, A., Séraphin, B., Serrano, L., 2001. Positive feedback in eukaryotic gene networks: Cell differentiation by graded to binary response conversion. EMBO J. 20 (10), 2528–2535.

Brabletz, Thomas, 2012. EMT and MET in metastasis: Where are the cancer stem cells? Cancer Cell 22 (6), 699–701.

Cherry, J.L., Adler, F.R., 2000. How to make a biological switch. J. Theoret. Biol. 203, 117–133.

Delbrück, M, 1949. Unités biologiques douées de continuité génétique. Colloques Internationaux du CNRS, Paris.

Dhooge, A., Govaerts, W., Kuznetsov, Yu.A., Meijer, H.G.E., Sautois, B., 2008. New features of the software MatCont for bifurcation analysis of dynamical systems. MCMDS 14 (2), 147–175.

Elowitz, M.B., Leibler, S., 2000. A synthetic oscillatory network of transcriptional regulators. Nature 403, 339–342–133.

Ferrell, Jr., James E., 2002. Self-perpetuating states in signal transduction: Positive feedback, double-negative feedback and bistability. Curr. Opin. Cell Biol. 14 (2), 140–148.

Fischer, Kari R., et al., 2015. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 527 (7579), 472–476.

Gardner, T.S., Cantor, C.R., Collins, J.J., 2000. Construction of a genetic toggle switch in Escherichia coli. Nature 403, 339–342–133.

Glass, Leon, 1975. Combinatorial and topological methods in nonlinear chemical kinetics. J. Chem. Phys. 63 (4), 1325–1335.

Goetz, Hanah, et al., 2020. A plausible accelerating function of intermediate states in cancer metastasis. PLoS Comput. Biol. 16 (3), e1007682.

Hanahan, Douglas, Weinberg, Robert A., 2000. The hallmarks of cancer. Cell 100 (1), 57–70.

Hoek, Keith S., et al., 2008. In Vivo switching of human melanoma cells between proliferative and invasive states. Cancer Res. 68 (3), 650–656.

Jiang, B., et al., 2021. Machine learning of genomic features in organotropic metastases stratifies progression risk of primary tumors. Nature Commun. 12, 6692.

Jin, X., et al., 2020. A metastasis map of human cancer cell lines. Nature 588 (7837), 331–336.

Jolly, Mohit Kumar, et al., 2017. EMT and MET: Necessary or permissive for metastasis? Mol. Oncol. 11 (7), 755–769.

Kalluri, Raghu, Weinberg, Robert A., 2009. The basics of epithelial-mesenchymal transition. J. Clin. Investig. 119 (6), 1420–1428.

Kauffman, Suart A., 1993. The Origins of Order: Self Organizationa nd Selection in Evolution. Oxford University Press.

Kohrman, Abraham Q., Matus, David Q., 2017. Divide or conquer: Cell cycle regulation of invasive behavior. Trends Cell Biol. 27 (1), 12–25.

Lee, Jiyoung, et al., 2014. Network of mutually repressive metastasis regulators can promote cell heterogeneity and metastatic transition. Proc. Natl. Acad. Sci. USA 111 (3), E364–73.

Lu, Mingyang, et al., 2013. MicroRNA-based regulation of epithelial-hybrid-mesenchymal fate determination. Proc. Natl. Acad. Sci. USA 110 (45), 18144–18149.

Mladinich, Megan, et al., 2016. Tackling cancer stem cells via inhibition of EMT transcription factors. Stem Cells Int. 2016, 5285892.

Pastushenko, Ievgenia, et al., 2018. Identification of the tumour transition states occurring during EMT. Nature 556 (7702), 463–468.

Pugh, Charles, 2015. Mathematical Analysis. Springer.

Pujals, Enrique R., Shub, Michael, Yang, Yun-Jung, 2020. Stable and non-symmetric pitchfork bifurcations. Science China Mathematica 63, 1837–1852.

Rajapakse, Indika, Smale, Steven, 2017. Mathematics of the genome. Found. Comput. Math. 17, 1195–1217.

Rand, David A., Raju, Archishman, Saez, Meritxell, Sigga, Eric D., 2021. Geometry of gene regulatory dynamics. Proc. Natl. Acad. Sci. USA.

Selvaggio, Gianluca, et al., 2020. Hybrid epithelial-mesenchymal phenotypes are controlled by microenvironmental factors. Cancer Res. 80 (11), 2407–2420.

Simeonov, Kamen P., et al., 2021. Single-cell lineage tracing of metastatic cancer reveals selection of hybrid EMT states. Cancer Cell 39 (8), 1150–1162.

Spencer, Sabrina L., et al., 2013. The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit. Cell 155 (2), 369–383.

Thiery, Jean Paul, Sleeman, Jonathan P., 2006. Complex networks orchestrate epithelial-mesenchymal transitions. Nat. Rev. Mol. Cell Biol. 7 (2), 131–142.

Thom, Rene, 1969. Topoligical models in biology. Topology 8, 313–335.

Thom, Rene, 1975. Structural Stability and Morphogenesis. WA Benjamin Inc.

Waddington, C.H., 1940. Introduction to modern genetics. Lond. R. Entomol. Soc. 14 (4-6), 82.

Zaritsky, A., et al., 2021. Interpretable deep learning uncovers cellular properties in label-free live cell images that are predictive of highly metastatic melanoma. Cell Syst. 12 (7), 733–747.

Zhang, Jingyu, et al., 2014. TGF-β-induced epithelial-to-mesenchymal transition proceeds through stepwise activation of multiple feedback loops. Sci. Signal. 7 (345), ra91.

Zheng, Xiaofeng, et al., 2015. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature 527 (7579), 525–530.