Applications of multisource data-based dynamic modeling to cell-cell signaling and infectious disease spreading

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Chapter 8

Final discussion

In this thesis, we developed mathematical models for understanding Notch signaling (**Part I**) and infectious disease spreading (**Part II**) based on multisource data analysis. In **Part I**, from Chapter 2 to Chapter 5, we analyzed the experimental data by ourselves and others related to Notch signaling and refined the existing dynamic models. The analysis of these models provides us new insights into the mechanisms of Notch signaling and its roles in development such as angiogenesis. For instance, the hetero-dimerization of Notch ligands Dll4 and Jag1 explains the opposing effects of Dll4 and Jag1 on angiogenesis. In **Part II**, including Chapter 6 and Chapter 7, we analyzed the multi-source data such as air quality index and population movement rate, which suggests the factors affecting the spread of pathogens in a population. Taking these factors into account, we proposed dynamic models and analyzed such models aimed at assessing existing interventions or suggesting new strategies against infectious diseases. For example, we predict that improving air quality can reduce the basic reproduction number of respiratory infection. Below, we first discuss the key insights and future developments on Notch signaling (**Part I**) and then continue to discuss our work on infectious disease modeling (**Part II**).

8.1 Cell-cell signaling

8.1.1 Summarizing discussion

Based on our experimental data, which suggests that Notch ligand can form dimers and ligand dimer mediates Notch receptor cis-inhibition in the same cell, we proposed

a new model of Notch signaling in Chapter 2, in which Notch receptor cis-inhibition is mediated by Notch ligand dimer rather than Notch ligand monomer as assumed by previous mutual inactivation model [81]. This mutual inactivation model is a cornerstone for understanding the mechanisms in Notch signaling [210] and has been extended into different variants [83, 85, 87, 42] to study biological questions related to Notch signaling. Different from the mutually exclusive interaction of Notch ligand and receptor predicted by mutual inactivation model, our new model predicts that Notch ligand and receptor can co-exist in large numbers within the same cell. This prediction is supported by the observations during *Drosophila* tissue patterning [79, 89, 90, 91, 92] and can be further tested experimentally. Overall, our model sheds new light on the understanding of Notch signaling and suggests a new way to investigate Notch signaling-related processes by controlling the formation of Notch ligand dimer. Moreover, it is interesting to compare our predictions with those of previous models on Notch-related morphogenesis.

Traditionally, Notch activity was thought to down-regulate the expression of Notch ligand Dll4 in endothelial cells during angiogenesis [41, 42, 106, 264]. Based on published experimental data [43, 48, 62, 110], we extended this understanding of Notch activity-mediated regulation of Dll4 expression in endothelial cells in Chapter 3. We proposed a new model where increasing Notch activity down-regulates Dll4 expression when the level of Notch activity is lower than a certain threshold, whereas increasing Notch activity up-regulates Dll4 expression when Notch activity is higher than the threshold. This model fills in several gaps between theoretical understanding and experimental observation of tip endothelial cell selection during angiogenesis. For example, tip cells usually have high levels of Dll4, we explained that increasing the expression of Dll4 in all adjacent endothelial cell limits tip cell formation in angiogenesis [257]. Our model recapitulates the tip endothelial cells selection in sprouting angiogenesis that was observed under various experimental conditions [48, 59, 260, 277] while traditional dynamic models fail to recapitulate these experimental data in at least one or several aspects. Overall, our study in this chapter further improves our understanding of the role of Notch signaling in angiogenesis, which could shed new light on the treatment of pathological angiogenesis in cancer.

In Chapter 4, we investigated the molecular mechanisms of opposing effects of Notch ligands Dll4 and Jag1 on sprouting angiogenesis. Our experimental studies as well as published experimental data present three biological features of Dll4 and Jag1 in Notch signaling: (1) Dll4 can self-associate to form homodimer that mediates Notch receptor cis-inhibition [47], whereas Jag1 alone behaves as monomer which is

insufficient to mediate the cis-inhibition of Notch; (2) Notch ligands Dll4 and Jag1 can heterdimerize when they are expressed in the same cell; (3) Notch receptor has higher affinity for Dll4 than that for Jag1 in trans-activation. In Chapter 4, we have integrated these interactions into the mathematical model that we had proposed in Chapter 3, and proposed an extended model including Jag1 dynamics. Mathematical analysis and numerical simulations of this extended model including Jag1 dynamics show that the Dll4-Jag1 hetero-dimerization coupling with the asymmetric trans-affinity of Notch for Dll4 and Jag1 leads to the *in vivo* observation that Jag1 promotes angiogenesis while Dll4 limits this process [43]. Furthermore, our model kills two birds with one stone. Using engineering micropattern of plated trans Jag1 and trans Dll4, Tiemeijer et al. [268] recently reported that Dll4, but not Jag1, elicited spatial control of endothelial sprouting. Our model faithfully recapitulates their published data, which is in agreement with their modeling research [268]. Boareto et al. [42] predicted that the reason for the opposing effects of Jag1 and Dll4 on angiogenesis should be that Notch signal up-regulates Jag1 expression and down-regulates Dll4 in endothelial cells, thus high production of Dll4 leads to alternating tip cells and stalk cells while high production of Jag1 leads to a homogeneous hybrid cell phenotype along a line of interacting cells. To date, however, this hybrid endothelial cell has not been confirmed experimentally. Different from their predictions, our research highlights the Dll4-Jag1 dimerization and the asymmetric affinity of Notch for two kinds of ligands, which is amenable to experimental validation.

In Chapter 5, we proposed a multi-scale dynamic model of vasculogenesis by integrating the ordinary differential equation (ODE) model that we have proposed in Chapter 4 into the Cellular Potts model (CPM) to investigate the effects of cell-cell signaling on vasculogenesis. Based on observations in the HMEC-1 cell culture system, we hypothesized two rules in the interactions between endothelial cells: (1) dispersed endothelial cells attract each other; and (2) contacting cells repel each other. Inspired by actin polarization in endothelial cells during angiogenesis [61], we assumed that cell motility during vasculogenesis is driven by cell polarization and the direction of polarization is determined by the cell-cell interactions. Following previous computational modeling [38], we introduced cell polarization into CPM cells and the cell-cell signaling described by ODE model affects the polarization activity in each CPM cell. The multi-scale model reproduces the vascular network patterning and recapitulates the morphodynamics of endothelial cells during *in vitro* vasculogenesis. Overall, we predict that the complex multi-cellular behaviour can emerge from simple cell-cell interactions. This modeling method not only provides research tools for vasculoge-

nesis, but can also be extended to other multi-scale development processes such as angiogenesis and lymphangiogenesis [417].

8.1.2 Outlook

The work on Notch signaling in this thesis shows that the interplay between experimentation and mathematical modeling advances our understanding of cell-cell signaling and its role in tissue development. Moreover, this reciprocal research paradigm, driven by both data and models, stands as a cornerstone methodology in mathematical biology. On the one hand, the increasingly abundant real data provide evidence to improve mathematical models; on the other hand, mathematical models and mathematical tools for analyzing data promote our understanding of biological systems and provide new insights into experimental directions or disease-specific treatments. Nevertheless, all mathematical models are imperfect necessarily [418]. The input of new data has the potential to refine or totally revolutionize the modeling for specific biological systems. For instance, our collaborators recently discovered that in addition to Notch ligands (see Chapter 2 and Chapter 4), Notch receptors themselves can form oligomers/dimers. The biological consequences of Notch receptor dimerization remain unexplored in current models, representing a future direction for our ongoing Notch-related modeling research.

In turn, the predictions based on mathematical models can inspire new experiments. For example, in Chapter 2 we have predicted that over-expressing Notch in cells does not affect the level of Notch ligand monomer in the same cell, a result that is required for our predictions of *Drosophila* tissue patterning. Although some indirect experiments currently support this prediction [79, 89, 90, 91, 92], direct evidence is lacking. In the future, this prediction result in *Drosophila* could be directly verified or falsified by analyzing Notch ligand level in Notch-overexpressing cells *in vitro* or *in vivo*. In Chapter 3 and Chapter 4 we have proposed dynamic models for endothelial cell fate decision in angiogenesis which recapitulate the observations of tip endothelial cell selection. A novel prediction is that the heterodimerization of Dll4 and Jag1 acts as a lever for opposing effects of Dll4 and Jag1 on angiogenesis. To verify this prediction, identifying the domain or site (e.g., EGF7 in Dll4, see Chapter 2) which controls heterodimerization of Dll4 and Jag1 is required. And then we can test the effects of mutant Dll4 and Jag1 that can not form hetero-dimer on angiogenesis using cell culture system [62, 325] and even *in vivo* experiments [43].

Although we have modeled Notch signaling and investigated its effect on tip cell

slelection in sprouting angiogenesis, the link between cell-cell signaling and multicellular motility during sprouting angiogenesis is not fully considered in this thesis. As a simplification, in Chapter 5 we have integrated our dynamic model for cell fate decision into the Cellular Potts model (CPM) to explain the negative effects of overexpressing Dll4 on vasculognesis [259]. It would be of interest to test if the same theoretical model could recapitulate sprouting angiogenesis and explain the observations such as cell rearrangements in sprouting angiogenesis [419]. In addition, the extracellular matrix (ECM) has been reported to play an important role in mediating cell differentiation [40], Notch signaling [420] and angiogenesis [328], but it is not included in the modeling of this thesis. Therefore, considering the cell-ECM interaction while modeling the cell-cell communication would further refine our dynamic models and improve our understanding of cell-cell signaling and angiogenesis.

8.2 Infectious disease spreading

8.2.1 Summarizing discussion

In **Part II** of this thesis, we developed dynamic models for infectious disease spreading based on multi-source data analysis. In Chapter 6, we first presented evidence for a causal relationship between respiratory infection and several environmental factors, where air pollution has a driving effect on the incidence of respiratory infection. And then we proposed a dynamic model with a particular focus on air pollution and respiratory infection transmission, where the incidence of respiratory infection is a function of air quality index (AQI). For air pollution, we modeled the dynamics of AQI with associated control measures by using a piecewise smooth function to reflect that an enhanced control strategy is implemented once AQI exceeds a certain level F_c, which yields a non-autonomous Filippov system. Mathematically, this study enriches the research content of non-smooth systems [355]. Particularly, we built a 'bridge' from the non-autonomous Filippov system to autonomous Filippov system, which has been applied by Zhou et al. [421] in analyzing a non-autonomous model for the transmission of West Nile Virus. Moreover, the theoretical analysis and numerical simulations of the model predicts that timely implementing control strategy against air pollution could effectively improve air quality and thereby reduce the incidence of respiratory infection, which could shed new light on the prevention and control of air pollution and respiratory infection.

In the presence of an infectious disease characterized by high infectivity and mor-

tality rates, implementing mobility restrictions may become necessary to impede the rapid dissemination of the disease [20, 381, 382, 195, 196, 197]. As the epidemic threat is reduced, it becomes critical to predict when and where travel restrictions can be lifted, and how to re-organize orderly travel movement. For this question, the previous modeling research only made an indirect prediction based on the idea of 'what if' [192, 193, 194]. For instance, what is the final size of an infection if 50% mobility restriction was lifted in the model? In Chapter 7, we developed a novel method to answer the 'reopen' question directly. We first give a necessary condition for reopening and design a practical algorithm to select the location and time of reopening. And then we designed a new algorithm to estimate the movement rate of population between the selected locations at the optimal time. As an example, we focused on the lockdown against COVID-19 in China and suggested a travel scheme between selected cities at the estimated optimal time. In contrast to previous 'what if' research methods, our study provides a new method which suggests some insights into how to lift the lockdown with minimal risk when a severe epidemic is under control.

8.2.2 Outlook

In **Part II** of this thesis, the work on infectious disease suggests several points to reduce the risk of infection and enriches the mathematical tools for investigating key questions such as optimal control [422] and mobility restrictions [423] in epidemiology. However, there are still some deficiencies in these works that need to be further improved and studied in depth.

(1) The mathematical model of respiratory infection assumed a homogeneous population. However, reported data show that the incidence of respiratory infection in children is higher than the incidence in adult [29] and the contact pattern of a population is age-dependent [27]. Analyzing a non-smooth epidemiological model with age structure is very challenging, but the result could shed new light on the precise prevention and control of infectious disease.

(2) The infectiousness of all infectious individuals is a constant in our mathematical modeling for infectious disease transmission. The mutation and evolution of pathogens [424] during epidemics has not been considered in **Part II** of this thesis. Although existing studies have investigated the evolution of pathogens in epidemics at the theoretical level [24], the research is difficult to combine with actual data [425]. How to develop dynamic models to quantify pathogenic mutations and long term epidemics needs to be explored further.

(3) The dynamics of immune response and vaccine effect decay [426] is important for understanding pathogen spread and predicting the effects of interventions against disease transmission [427]. By integrating such temporal dynamics of vaccine efficacy, we could develop the mathematical model proposed in Chapter 7 into a multi-scale dynamic model of infectious disease transmission. Analyzing such multi-scale epidemiological model could shed new light on designing cross-biological scale joint treatment programs against infectious diseases.

All in all, in this thesis we have shown how multisource-based dynamic modeling can be integrating in analyzing complex biological systems. To be most effective, such studies introduce a series of assumptions to simplify the model. In future work the role of mathematical modeling in biological research could be generalized and further combined with real data.