MODELLING OF MACROPHAGES INTERACTIONS IN BREAST CANCER BY PARTIAL DIFFERENTIAL EQUATIONS

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Especially to my beloved parents,
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ABSTRACT

The signalling interaction between tumor cells and macrophages will form spontaneous aggregation that causes tumor spreading. Tumor cells and macrophages interchange their respective signals which results a paracrine and autocrine signalling loop. This interaction process can be represented by mathematical model in the form of chemotaxis and reaction diffusion equations. The existing models that consider paracrine signalling loop alone or with the inclusion of paracrine and autocrine signalling loop had been developed by assuming linear signals production. However, this assumption does not give a better representation on the signal dynamics where it is supposed to be in nonlinear form that saturate with increasing cell densities. Therefore, in this research, two existing interaction models are improved by considering the nonlinear form of signals production. Besides, another new interaction model is also developed based on the facts that tumor cells release enzyme during the signaling interaction to penetrate the surrounding tissues. The stability analysis is conducted on three separated models to investigate the condition for spontaneous aggregation. Each of these conditions then are validated using numerical simulations. Stability analysis shows that for all models, the formation of aggregation could be determined by the parameter that represents the secretion and degradation rates of signals together with chemotaxis rates towards signals. However, the inclusion of autocrine signalling loop in the second model increase the possibility of the aggregation. While in the third model, an additional parameter that represents the secretion and degradation rates of enzyme as well as chemotaxis rates towards them could also determine the formation of the aggregation. By numerical simulations, the results are in agreement with the stability analysis obtained for each of the interaction models. Besides, cell clusters that result from the aggregation will be merged to the other cells cluster due to the “effective attraction” between them. Reducing the production rates of signal or chemotaxis rates towards signals or increasing degradation rates of signal is required to prevent aggregation. The same changes towards enzymes will give the same result on preventing the aggregation. These valuable suggestions are crucial for medical experts during treatments.

ABSTRAK

Interaksi secara isyarat di antara sel tumor dan makrofaj akan membentuk aggregasi secara spontan yang mengakibatkan tumor merebak. Sel tumor dan makrofaj saling bertukar-tukar isyarat yang kemudiannya menghasilkan gelung isyarat parakrin dan gelung isyarat autokrin. Model matematik bagi proses interaksi ini boleh dibentuk menggunakan kemotaksis dan persamaan reaksi serapan. Model sedia ada yang melibatkan gelung isyarat parakrin sahaja atau bersama gelung isyararat autokrin telah dibentuk dengan andaian bahawa penghasilan isyarat adalah secara linear. Walaubagaimanapun, andaian ini tidak menggambarkan dinamik isyarat yang baik kerana penghasilan isyarat seharusnya dalam bentuk tidak linear yang mana tepu apabila ketumpatan sel bertambah. Oleh itu, dalam kajian ini, kedua-dua model yang sedia ada diperbaiki dengan mempertimbangkan bentuk tidak linear untuk penghasilan isyarat. Selain itu, satu interaksi model baharu yang lain telah dibentuk juga berdasarkan fakta bahawa sel tumor merembeskan enzim ketika interaksi secara isyarat itu berlaku untuk menembus tisu sekeliling. Analisis kestabilan dijalankan pada ketiga-tiga model tersebut untuk mengkaji syarat pembentukan aggregasi secara spontan. Setiap syarat tersebut kemudiannya disahkan menggunakan simulasi secara berangka. Analisis kestabilan untuk kesemua model menunjukkan bahawa pembentukan aggregasi boleh ditentukan oleh parameter yang mewakili kadar penghasilan dan penguraian isyarat-isyarat bersama dengan kecenderungan sel terhadap isyarat-isyarat tersebut. Walaubagaimanapun, penglibatan gelung isyarat autokrin dalam model kedua meningkatkan kebarangkalian untuk aggregasi berlaku. Manakala model ketiga menunjukkan pertambahan parameter yang mewakili kadar penghasilan dan penguraian enzim beserta kecenderungan sel terhadapnya juga boleh menentukan pembentukan aggregasi. Simulasi secara berangka telah mengesahkan keputusan yang telah diperolehi daripada analisis kestabilan bagi setiap interaksi model. Selain itu, pembentukan sel kluster hasil daripada aggregasi akan bergabung dengan sel kluster yang lain disebabkan “penarikan berkesan” di antara mereka. Mengurangkan kadar penghasilan atau kecenderungan terhadap isyarat-isyarat yang terlibat atau menambah kadar penguraiannya diperlukan dalam mencegah aggregasi. Perubahan yang sama juga perlu dilakukan kepada enzim untuk mencegah aggregasi. Cadangan yang sangat berguna ini penting kepada pakar perubatan semasa merancang perawatan.

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LIST OF ABBREVIATIONS

CSF-1	–	Colony stimulating factor-1
CSF-1R	–	Colony stimulating factor-1 receptor
EGF	–	Epidermal growth factor
EGFR	–	Epidermal growth factor receptor
ECM	–	Extracellular matrix
MDE	–	Matrix-degrading enzyme
MMP	–	Matrix metalloproteinase
KS	–	Keller and Segel
TAMs	–	Tumor associated macrophages
DLIT	–	Diffuse Luminescent Imaging Topography
cAMP	–	cyclic adenosine monophosphate
uPA	–	urokinase plasminogen-type activator
Targit	–	Targerted intraoperative radiotherapy
EBRT	–	External beam radiotherapy
DLIT	–	Diffuse Luminesent Imaging Tomography

LIST OF SYMBOLS

∇	–	Differential operator
M	–	Density of macrophages
T	–	Density of tumor cells
C	–	Concentration of CSF-1
E	–	Concentration of EGF
μ	–	Random motility of cells
s_1, s_2, b_1, b_2	–	Secretion of signals
h_1, h_2	–	Average density of cells
$\chi_1, \chi_2, \chi_3, \chi_u$	–	Chemotaxis coefficient
D	–	Diffusion of signals
$\gamma_1, \gamma_2, \gamma_3$	–	Degradation of signals
ξ	–	Small perturbation of macrophages
η	–	Small perturbation of tumor cells
M_0, T_0	–	Amplitude of perturbation
q	–	Wavenumber of perturbation
σ	–	Linear growth rate of perturbation
x_{rand}	–	Random number
U	–	Concentration of MDE
V	–	Density of ECM
β	–	Digestion rate of ECM
D_u	–	Diffusion of matrix degrading enzyme
s_3	–	Secretion of matrix degrading enzyme
γ_3	–	Degradation of matrix degrading enzyme

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancer continues to be an enormous global health problem, accounting for an estimate of 8.9 million deaths worldwide in 2016. The number is expected to increase significantly over 10 years if there is lack of effort to improve existing treatments. This issue arises due to the challenges faced by experts to deal with the heterogeneity of cancer itself. Cancer shows distinct characteristics and profiles within a patient's tumor and among tumors from different patients which can complicate diagnosis and therapy. The best way to overcome this challenges is to understand the characteristic and behaviour of cancer.

In general, there are more than 100 types of cancer that affect human. Breast cancer is the most common diagnosed cancer types and a leading killer among women across the globe [1–3]. It is also possible to occur in men and children, however this is rare. Breast cancer can begin in many different areas of the breast such as ductal (passage for milk) and lobule (stores milk) which can be non-invasive, invasive and metastatic. Non-invasive cancer do not spread to nearby tissue while invasive cancer can move out and spread from nearby breast tissues. If the cancer cells break free from the primary site and migrate to other parts of the body, it is considered as metastatic and can lead to death. Most previous studies aim to prevent metastatic event to reduce the death risks of breast cancer patients.

For human survival, the immune system plays an imperative role against cancer [4–6]. Macrophage is one of its division, and it stands out as the most multifunctional among other types of innate immune system [7–12]. It can perform different functions depending on the environmental cues. Tumor cells taking this advantage, manipulate the macrophages to escape themselves from being detected as foreign cells by creating

a signalling interaction with macrophages. This will induce the motility of tumor cells that results a spontaneous aggregation with macrophages which results in a migration to nearby tissues and form cancer in new sites. This situation often relates with a poor prognosis in several types of cancer including breast cancer [7–12].

The existence of interaction between marophages and tumor cells is considered the most crucial event [10–13]. Both tumor cells and macrophages can interact by interchange their respective signals that results spontaneous aggregation for migration [10, 13–15]. During migration, tumor cells also try to break down the extracellular matrix (ECM) using mediators called matrix degradative enzymes. This method of invasion by tumor cells are the hallmarks of metastasis which causes death among breast cancer patients. Thus, the main focus in this research is to model the interaction and invasion process between macrophages and tumor cells using mathematical knowledge, known as a system of partial differential equations.

1.2 Background of the Study

Macrophage is derived from circulating precursor called monocytes in the blood vessels which comprises an approximate 2-10% populations of white blood cells. Generally, macrophages are essential component for host defense mechanism against pathogens [16–19]. They are also responsible to stimulate the growth of tissues, secrete molecules for angiogenesis (formation of blood vessels), engulfment of the dead cells and matrix remodeling (tissues compartment that defines shape, characteristic and dimensions of organs) [10, 15, 20].

Based on the diverse role of macrophages, it may act as a promoting or suppressing role in their immunity behaviour during immune response depending on the environmental cues. This fact further suggests that they may undergo classical M1 activation or alternative M2 activation [21–27]. M1 macrophages are pro-inflammatory that have the ability to kill pathogens while M2 macrophages are anti-inflammatory that downregulate the inflammatory response, promote angiogenesis and remodelling of tissues. In tumor environment, M1 macrophage are tumoricidal compared to M2 macrophage which have a weak tumoricidal capability.

In tumor microenvironment, macrophages are often referred as tumor associated macrophages, TAMs [28]. These macrophages are closely related to M2

type macrophages which tends to perform trophic and immunosuppressive rather than immunity behavior. TAMs are recruited by tumor cells using variety of growth factors and cytokines such as monocyte/macrophage chemoattractant protein-1, MCP-1/CCL2 [29]. MCP-1 possess chemotaxic activity for monocytes and T lymphocytes via its receptor called CCR2. Several cancer including mammary, ovarian, pancreatic, prostate and renal cancer have been shown that there is correlation between the concentration of MCP-1 with the leukocytes [30].

In breast cancer, the infiltration of macrophages result in a poor prognosis of the disease [9, 10, 12, 28]. The infiltration trigger the interaction to exist between tumor cells and macrophages. Qian and Pollard [11] have reviewed several studies on interaction between tumor cells and macrophages. The interaction initiated by tumor cells which secrete colony stimulating factor-1, CSF-1 that received by CSF-1 receptor, CSF-1R on macrophages. This will trigger macrophages to secrete Epidermal Growth Factor, EGF that can be received by its receptor, EGFR on the tumor cells. Each cell type responds to the signal from the other type by chemotacting towards a higher concentration gradient. This interaction will create a paracrine signaling loop which will results a spontaneous aggregation and leads to cooperative migration for metastasis. Recent studies also reveal that tumor cells have their own receptor for their own signal which then create another loop called autocrine signaling loop.

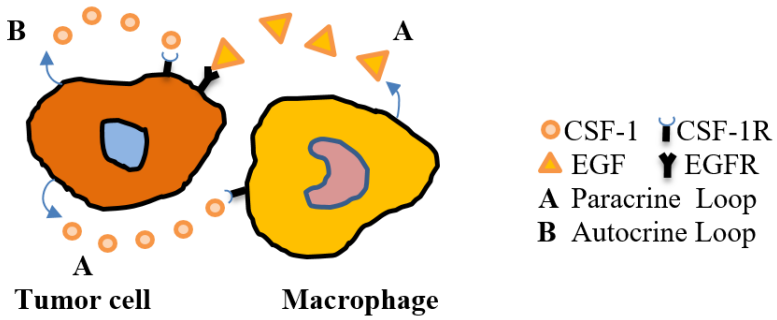

Figure 1.1: Signalling communication between macrophage and tumor cell.

During the interaction, there is another crucial event that need to be highlighted. As previously mentioned, tumor cells migrate in the sense of EGF released by macrophage. In order to move towards the concentration gradient, tumor cells need to pass through the surrounding tissue or extracellular matrix (ECM) [10, 14, 31]. This invasion process is facilitated by matrix degrading enzymes (MDEs), such as Matrix Metalloproteinases (MMPs) released by tumor cells. Since ECM components are made up of many macromolecules that have different physical and biochemical properties,

the MDEs are required to break down the component in order for tumor cells to invade the surrounding tissue.

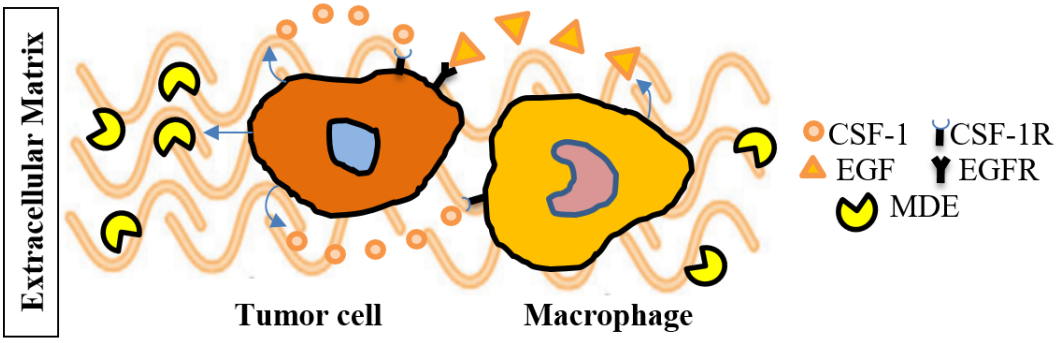

Figure 1.2: Signalling communication between macrophage and tumor cell in extracellular environment.

Based on the interaction, the movement of tumor cell and macrophages in response to chemical gradient can be referred as chemotaxis. It has great significant as proven in previous studies due to its critical role in a wide range of biological phenomena. Keller and Segel [32] first used partial differential equations to model the interactions of chemotactic cells (slime molds) and its secreted attractant (cAMP). Many researchers utilized their model since it is able to capture key phenomena, is easy to understand and analyzed analytically and numerically. For example, Lauffenburger and others [33, 34] motivated by the KS model to describe the inflammatory response of leukocytes to bacterial infection. Luca *et al.* [35] also investigated whether the chemotactic aggregation of microglia may contribute to senile plaques during development of Alzheimers diseases.

The earliest work that study the interaction between macrophages and tumor cells in breast cancer was done by Knutsdottir *et al.* [36]. They used a system of partial differential equations that consists of chemotaxis and reaction-diffusion equations to model the signalling interactions between both cells. Elitas and Zeinali [37] then, inspired by this work, developed another mathematical model in different sites. They developed a mathematical model of movement and binding between macrophage and glioma cells in the brain. Both models use the same type of signalling molecules, namely colony stimulating factor-1 (CSF-1) and epidermal growth factor (EGF).

In Knutsdottir *et al.* [36] works, they considered the secretion terms for both signals have linear relationship with the density of cells. In other words, the production

of both chemical signals are increases when the density of cells increases. However, this assumption is too simplistic and does not capture the true signal dynamics. Hillen and Painter [38] suggest that the production of chemical signals are supposed to saturate with increasing cells density which can be represented mathematically in the form of nonlinear functions. A number of chemotactic models also used this term that can be found in Maini *et al.* [39] and Myerscough *et al.* [40].

Besides, the model proposed by Knutsdottir and their co-workers does not consider the interaction involving ECM. In real situation, the interaction involved is not only between tumor cells and macrophage, but also ECM. This inclusion is motivated from pioneering work carried out by Anderson *et al.* [41]. They are among the earlier researchers who have developed a mathematical modelling using continuum and discrete model that describe the invasion of host tisue by tumor cells through ECM which is facilitated by MDEs. Afterwards, the model is improved by other researcher considering various assumptions to enhance understanding about the interaction involving tumor cell and ECM. Chaplain and Lolas [42] are one of them, focused on continuum model that consider chemotaxis and haptotaxis mechanism by tumor cells and remodelling of ECM after degrading process. Their work was then extended by Tao and Cui [43] where they assume nonlinear density-dependent term for chemotaxis and haptotaxis in tumor cells. Ramis-Conde *et al.* [44] take different approach by proposing a hybrid discrete-continuum two-scale model to study the early stage of tumor cell and its ability to invade the surrounding ECM.

1.3 Statement of the Problem

In breast cancer, the infiltration of macrophages lead to motility of tumor cells. Tumor cells and macrophages communicate by signalling to each other to form aggregation that results in migration. Previous researcher have proposed model using chemotaxis and reaction diffusion equations to illustrate the interaction between macrophages and tumor cells with their production of signalling molecules, EGF and CSF-1 respectively. The model assume each production of signals have linear relationship with the density of each cells. However, this assumption does not give a better representation about the true dynamics of signals. The production of chemical signals are supposed to saturate with increasing cells density. This term is in the form of nonlinear functions which have been widely used in a several number of chemotactic models.

Besides that, the existing model published does not consider the involvement of ECM and MDE during the interaction between macrophages and tumor cells. In real situation, tumor cells need to penetrate ECM for migration towards higher concentration gradients of EGF using MDE. This interaction also can be known as invasion process by tumor cells. Many mathematical models have been developed to illustrate this invasion process. Thus, this could help this research to develop new interaction model to study the effect of the inclusion of ECM and MDE in the interaction between tumor cells and macrophages.

1.4 Objectives of the Study

The objectives of this research are as follows:

1. To modify the mathematical model of interaction between macrophage and tumor cell by considering nonlinear functions for signals production in each cases:
    - Paracrine signalling loop.
    - Paracrine and autocrine signalling loop.
2. To modify the mathematical model of interactions between macrophages and tumor cells with the inclusion of extracellular matrix (ECM) and matrix degrading enzyme (MDE) by considering linear functions for signals production in paracrine signalling loop cases.
3. To determine the conditions for cell aggregation by performing stability analysis of the interaction models.
4. To validate the stability analysis of the interaction models and observe the behaviour of the aggregation by performing numerical simulations.

1.5 Scope of the Study

System of one dimensional partial differential equation that consists of chemotaxis and reaction-diffusion equation is used to develop a mathematical model for interaction between macrophage and tumor cells in breast cancer. In this research, the environmental cues involved in the interaction are epidermal growth factor, EGF, colony stimulating factor-1, CSF-1 and matrix degrading enzyme, MDE.

There are two methods to achieve the objectives which are stability analysis and numerical simulations. In stability analysis, the perturbation method is use to linearize the interaction models. In this research, small perturbation is introduced to the models which it is in the form of exponential type. While in numerical simulations, a built in PDE solver called *pdepe* in MATLAB software is used to solve the interaction models to observe the aggregation behaviour. This solver is designed to solve parabolic and elliptic systems with the constraints that there must be at least one parabolic equation given.

There are limitation to validate the interaction models with the real data. Since the human breast cancer data are not possible to obtain in local medical laboratory, the results obtained are only compared with the previous researcher. In previous researcher, the results obtained are validate with the experimental findings in the literature that conducted on mice. Although it compared with mice, their results are still relevant. This is because in clinical research, it is not suitable to experiment or test directly on human being. Since mice is the most suitable animal that share almost the same biological characteristics with human, thus the findings are practical to be applied in human.

1.6 Significance of the Study

Macrophage is one of the earliest immune response that reaches the tumor site to prevent tumor progression. However, the experimental studies reveal that macrophages are able to promote the progression of tumor in certain types of cancer. In the mathematics field, various mathematical models were developed based on the tumoricidal capability of immune system. This research should inspire other mathematical researchers to keep update about the new biological findings related to tumor immunology obtained from experimental studies. Thus, they can contribute either in developing new mathematical model or improve the existing model.

Besides, this research highlights the interactions between macrophages and tumor cells results in spontaneous aggregation that leads to tumor spreading. Through mathematical model, it is possible to determine the condition for aggregation so that several actions can be proposed to medical experts during drug treatments. This effort is aim to prevent tumor progression thus can reduce the death risk among cancer patients.

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