MATHEMATICAL MODELING AND SIMULATION IN AN INDIVIDUAL CANCER CELL ASSOCIATED WITH INVADOPODIA FORMATION

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A dissertation submitted to
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ABSTRACT

The degradation of the extracellular matrix (ECM) is driven by actin-rich membrane protrusions called invadopodia, which leading to the cancer cell invasion across the surrounding tissue barriers. Signaling pathways through ligand and membrane associated receptor bindation are vital point in order to enhance the actin polymerization activities that push the membrane of migrating cells. The results presented by Saitou et. al, [36] are contradict to this fact since actins are not only pushed, but also diffused beyond the cell membrane into the ECM region. Hence, in this study, we considered mathematical modeling for an individual cancer cell. We investigated one-dimensional Stefan-like problem of the signal process, (CM-I-B) and treated the cell membrane as a free boundary surface to separate any activity happen in intra- and extracellular regions. An approximation problem, (CM-I-C) is introduced by transforming the Stefan-like problem into an initial-boundary value problem for the signal equation with penalty term. The velocity concerning the movement of the free boundary is then calculated by the integration of the penalty term. The auxiliary problem is solved numerically using finite-difference scheme, (CM-I-C$'$) for the above integrated penalty method, [21]. Two convergences of CM-I-C and CM-I-C$'$ into CM-I-B are investigated by taking ε and δx goes to 0, respectively. Our results showed a good agreement with the other known fixed domain method for the free boundary positions and the signal distributions.

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CHAPTER 1

INTRODUCTION

1.1 Cancer Cells

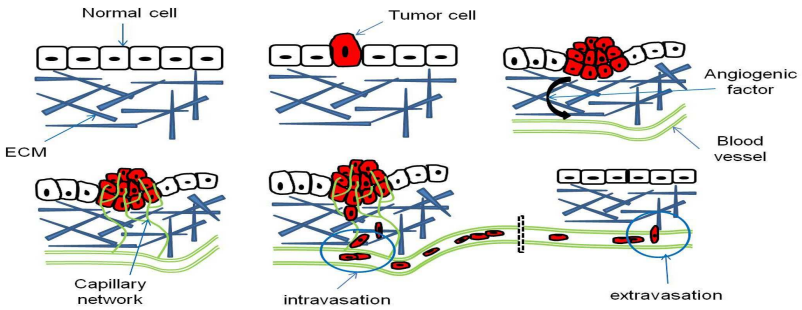

Figure 1.1: Key stages of the cancer cell invasion including two key hallmarks of angiogenic and metastasic cascades in order to develop the secondary tumor, [12].

Formation of a tumor cell begins by an individual normal cell in a tissue or an organ in the human body. After some mutations in their key genes, this cell is transformed into a cancer cell. The dissimilarity between a normal and a cancer cell is the potential of a cancer cell to escape from its body hemeostatic mechanisms that leads to proliferation. Furthermore, a cluster of cancer cells formed by some divisions in a cancer cell. Further growth and proliferation will enhance the cluster into avascular state of cancer cells where the size is contains approximately 10^6 cells. At this stage, avascular cancer cell cannot grow further and they merely able to receive nutrients and remove waste products. They also do not harm; in other words, make a tiny or no damage to the host tissue because the avascular cancer cell is relatively small, remain localized at one spot and do not spread, [3].

Avascular cancer cell experiences closed and recurrent activities, and they need to grow further in order to enlarge and establish their colony. For that reason, they will go through two crucial processes, famously known as angiogenesis and metastasis. Through the angiogenesis process, cancer cells secrete angiogenic factors such as vascular endothelial growth factor (VEGF) to instigate proteins located in the neighboring blood vessel to break down the basement membrane. Consequently, endothelial cells are allow to migrate and invade to the extracellular matrix (ECM) region. In the beginning, VEGF activates the endothelial cells to express the proteins, necessary to allow the development of new blood vessels that leading to the formation of loops and branches into the tumor. From these branches, more sprouts are formed and the whole process repeated until capillary network that connected the blood vessel and avascular cancer cell is produced. Eventually, avascular cancer cell have the opportunity to receive extra nutrients from blood circulation system that required for further growth, [4].

There is a possibility for each cancer cell to find their way to enter the blood circulation system and transported to the other tissues through the blood and lymph. Here, another key hallmark which is metastasis process started. In the early process, cancer cells try to escape from the avascular state where here and henceforth we named it as a primary cancer. Local degradation of surrounding tissue or ECM by potential cancer cells inspire a pathway to enter the blood circulation system. Invasion of cancer cells into a blood or lymphatic vessel through the basal membrane is known as intravasation. Cancerous cells need to survive in the journey throughout the circulation system until they reach at some places or distant sites in the body. In the period of time, they need to escape from the blood circulation system (extravasation) and must establish a new colony (secondary tumor) in the new organ. Hence, this completes the full blown of metastasis for a tumor cell, [8].

Here, we summarize several key stages for a cancer cell invasion including angiogenesis and metastasis cascades in order to develop secondary tumor, which are;

(i) growth of a primary tumor,

(ii) recruitment of new blood vessels by angiogenic growth factors,

(iii) formation of capillary network that connects the primary tumor and blood vessel,

(iv) cancer cells ready to escape from the primary tumor,

(v) local degradation of ECM by invasive cancer cells,

(vi) cancer cells enter the blood circulation system (intravasation),

(vii) survival of cancer cells along the journey in the blood circulation system,

(viii) cancer cell escape from the blood circulation system (extravasation),

(ix) establish a new colony in other sites of new organs,

(x) formation of a secondary tumor.

1.2 Research Background

Cell movement is important for its own reason. On a good side, white blood cells swim to heal cuts. On the other side, tumor cells travel and invade the surrounding tissue or extracellular matrix (ECM) to form the secondary tumor known as metastasis, which is the main cause of mortality among cancer patients, [39]. Tumorigenesis is a complex multi-step process that results from genetic changes and cause malignant transformation of normal cells, [8]. A summary of the characteristics common to many cancers is

(i) abnormal signal transduction resulting in uncontrolled cell proliferation,

(ii) loss of apoptosis or programmed cell death,

(iii) angiogenesis leading to the enhanced blood supply of tumors, and

(iv) tissue invasion and metastasis permitting spread of the cancer.

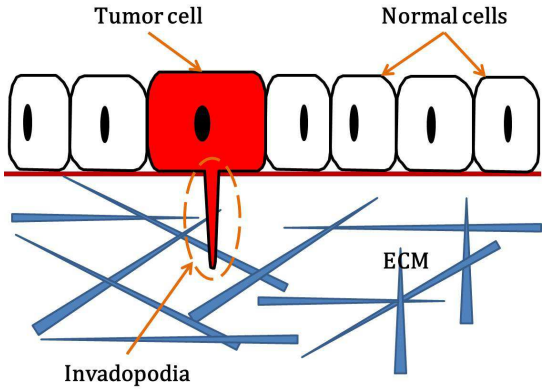

Figure 1.2: Movement of a cancer cell is driven by invadopodia, which engenders migratory pathways through the ECM.

Concentrating on the fourth feature, proteolytic deterioration of the ECM is a vital phenomenon in tumor invasion and metastasis, [1], [2]. This issue is driven by actin-rich protrusions of the plasma membrane or known as invadopodia. Over the pass decade, many works had been carried out in order to understand how invadopodia contributes in degrading the surrounding tissue barriers that lead to the cancer cell migration, [26], [34].

Mathematical approaches using continuous and discrete models, particularly by considering partial and ordinary differential equations in tumor growth take the role in the relationship between mathematics and oncology which is a branch of medicine, [3], [4], [8], [9], [23], [31], [41]. Furthermore, investigation on the cancer cell invasion through invadopodia formation have been considered by [36] in the mathematical point of view.

1.3 Invadopodia Formation

Invadopodia are subcellular structure found in invasive cancer cells uniquely formed by metastatic carcinoma cells. Formation of invadopodia and ECM degradation activity involves the coordination of many cell biological processes including ligand and epidermal growth factor receptor (EGFR) signaling, actin cytoskeletal

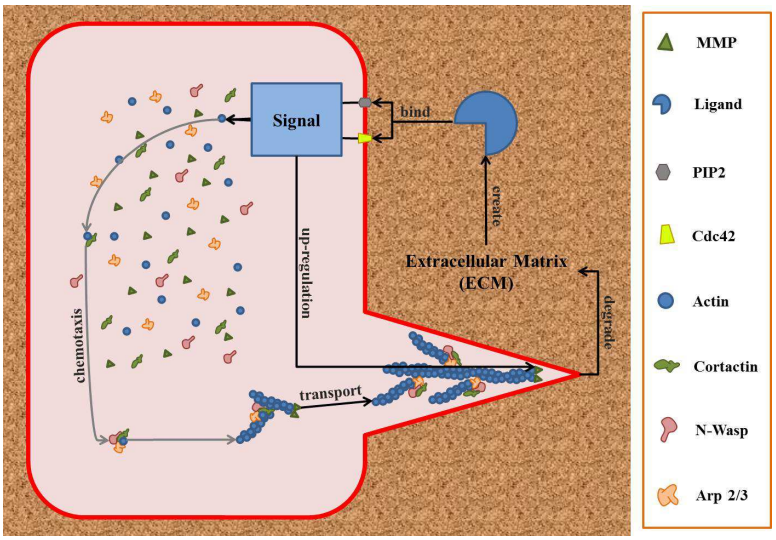

Figure 1.3: Feedback loop of invadopodia formation is driven by key-signal transduction.

polymerization and reorganization. These lead to physical force towards membrane surface and MMP localization, activation and secretion, [7], [16], [45].

Basically, binding of EGFR and ligand such as transforming growth factor alpha (TGF-α) plays an important role in the formation of the invadopodia, which activates a signaling pathway for actin branching and matrix metalloproteinases (MMPs) regulation, [36]. Branched actin activators are crucial for invadopodia formation which, for instance the activation of neuronal WiskottAldrich Syndrome protein (N-WASP) is essential for invadopodium maturation, [42].

During precursor formation, cortactin, Tks5, N-WASp, cofilin, and actin related protein 2/actin related protein 3 (Arp2/3) form a domain involving cortactin-Arp2/3-N-WASp binding complex. Tks5 is thought to promote the formation of invadopodium precursors when binding to phosphatidylinositol (3,4)-bisphosphate or PI(3,4)P2 on the ventral cell surface plasma membrane, [7].

Actin polymerization involves several stages including activation, nucleation, elongation and annealing. In the early step, a guanine-nucleotide exchange factor (GEF) such as Fgd1 activates the Rho GTPase cell division cycle 42 (Cdc42)

to regulate various factors of intra-cellular actin dynamics such as controlling rearrangements of membrane cytoskeleton, replication activation, secretion of membrane trafficking, cell cycle progression and tumorigenic transition, [14].

Then nucleation is a process of accumulating actin monomers (G-actin) to form a comparatively substantial core or nucleus which can hold the addition of other G-actin at both ends leading to the development of double helix filamentous polymer or F-actin. Nucleation is magnified by the complex interaction of adenosine triphosphate (ATP)-actin with adenosine diphosphate (ADP)-actin, [29]. Besides, elongation denotes association and dissociation of G-actin at both ends of F-actin. These capping proteins are attached into their positions by conformational changes or annealing. Polymerization predominantly takes place at the barbed end of the filament, otherwise depolymerization at the other side of pointed end, [33], [7].

The structural polarity of F-actin during actin assembly has implications on the rate and direction of filament growth at opposite ends of the actin filament. The critical concentration (CC) for the pointed end is larger than for the barbed end, [43]. Polymerization only happens if the density of G-actin exceeds CC, which concentration of monomers coexisting with polymer at the steady state of polymerization. In other words, if the concentration of G-actin is below CC, G-actin fail to polymerize and remain as a monomer. This process of association and dissociation is called treadmilling of actin, [32].

One of the previous studies showed that actin polymerization is regulated by cortactin, and therefore regulate the secretion of MMPs during the formation of invadopodia, [11]. One of the MMPs called membrane type-1 matrix metalloproteinase (MT1-MMP) is a protease involves in an invasion maneuver of tumor cells. It is normally found near to the membrane surface of invadopodia. During the ECM degradation, MT1-MMP activates secretive basal membrane enzyme called matrix metalloproteinase-2 (MMP2) to degrade the basal membrane made by collagen IV, [37], [38].

The process of shedding is a big picture for the basement degradation by MMP2. Binding of the MT1-MMP to the other molecule called TIMP

metallopeptidase inhibitor 2 (TIMP2) is important for the later formation of complex (MT1-MMP)-TIMP2-(pro-MMP2)-(MT1-MMP). Once this complex is achieved, other MT1-MMP which is free from TIMP2 cleaves the binding between TIMP2 and pro-MMP2 and activates the pro-MMP2 to degrade the mesh of fibrous proteins of the ECM, [15], [40].

1.4 Objectives and Scope

The objective of the study is to investigate the behavior of an individual cancer cell which invades to the surrounding tissue theoretically by solving the following problems:

(i) Application of integrated penalty method in one-dimensional signal transduction during the formation of invadopodia.

(ii) Convergences of an approximation model for one-dimensional signal transduction with integrated penalty to the original model.

(iii) Validation of the results obtained in (i) by using fixed domain method.

1.5 Thesis Organization

This thesis consists of four chapters. In Chapter 1, we have reviewed the mechanisms in the formation of invadopodia, and the objectives and scope of this research. In Chapter 2, there are two sections presented specifically for research gap opportunity and new-enhanced modeling including the cancer cell model for one-dimensional problem.

In Chapter 3 and Chapter 4, we have discussed two types of numerical method to solve our cell model which are integrated penalty (IPM) and fixed domain methods (FDM), respectively. In Chapter 3, we presented a mathematical modeling for one-dimensional Stefan and cancer cell models. Convergence analyses for cell model are proved to support the reliability of IPM.

On the other hand, Chapter 4 focused on providing good data for the validation purposes. For most cases, results from IPM are compared to the results obtained from FDM by considering similar conditions.

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