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Mathematical model of macrophage-facilitated breast cancer cells invasion

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HIGHLIGHTS

- We model paracrine and autocrine signalling between breast tumor cells and macrophages.
- Analytical results are confirmed with discrete and continuum model simulations.
- Motility of breast tumor cells can be eliminated by changing certain model parameters.
- We identify parameters responsible for the observed tumor cell to macrophage ratio.
- Sensitivity of tumor cells to autocrine signalling affects their ability to migrate.

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ABSTRACT

Mortality from breast cancer stems from its tendency to invade into surrounding tissues and organs. Experiments have shown that this metastatic process is facilitated by macrophages in a short-ranged chemical signalling loop. Macrophages secrete epidermal growth factor, EGF, and respond to the colony stimulating factor 1, CSF-1. Tumor cells secrete CSF-1 and respond to EGF. In this way, the cells coordinate aggregation and cooperative migration. Here we investigate this process in a model for *in vitro* interactions using two distinct but related mathematical approaches. In the first, we analyze and simulate a set of partial differential equations to determine conditions for aggregation. In the second, we use a cell-based discrete 3D simulation to follow the fates and motion of individual cells during aggregation. Linear stability analysis of the PDE model reveals that decreasing the chemical secretion, chemotaxis coefficients or density of cells or increasing the chemical degradation in the model could eliminate the spontaneous aggregation of cells. Simulations with the discrete model show that the ratio between tumor cells and macrophages in aggregates increases when the EGF secretion parameter is increased. The results also show how CSF-1/CSF-1R autocrine signalling in tumor cells affects the ratio between the two cell types. Comparing the continuum results with simulations of a discrete cell-based model, we find good qualitative agreement.

1. Introduction

In breast cancer, the presence of macrophages at the tumor site is related to poor prognosis (Condeelis and Pollard, 2006; Lewis and Pollard, 2006). This is surprising since macrophages are a part of our immune system. However, studies have shown that macrophages play various roles in tumor development and progression, one of which is to increase tumor cell motility. Tumor cells and macrophages in close proximity communicate via a short-ranged chemical signalling loop based on epidermal growth factor, EGF, secreted by macrophages, and colony stimulating factor 1, CSF-1, secreted by tumor cells (Lin et al., 2001, 2002; Goswami et al., 2005; Condeelis and Pollard, 2006; Lewis and Pollard, 2006; Wyckoff et al., 2007; Beck et al., 2009; Patsialou et al., 2009). This signalling results in aggregation (van Netten et al., 1993), so that tumor cells migrate alongside macrophages towards blood vessels or surrounding tissues and organs (Wyckoff et al., 2007). Extravasation (escape out of a blood vessel) results in metastasis, the formation of secondary tumors, a primary cause of death in breast cancer patients. Hence, limiting or eliminating tumor cell motility is a crucial part of cancer treatments. Experiments have shown that when the number of macrophages is decreased at breast

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cancer sites, tumor progression is slower and fewer cells are able to metastasize, resulting in increased survival rates (Lin et al., 2001). Here we use mathematical modelling to examine interactions between tumor cells and macrophages in a nutrient-rich *in vitro* situation.

In order for the tumor-macrophage interactions to result in group migration, the cells must have a net tendency for aggregation. By aggregation, we mean the tendency of the system to develop a nonuniform spatial distribution where cell clusters form. The density of such clusters is generally well-elevated over background densities, though we do not claim a specific density or size in order to call such a cluster "an aggregate". Some clues about the underlying process are provided by quantitative measurements. For example, in Wyckoff et al. (2004) it is found that the ratio of tumor cells to macrophages is 3 to 1 in experiments conducted in mice where cells were collected into micro-needles containing EGF. In Patsialou et al. (2009) a similar experiment is conducted using a human breast cancer cell line and the ratio between tumor cells and macrophages increases to 15 to 1. In this paper we explore how features of the paracrine signalling loop contribute to this tendency. Principally, we ask the following questions:

- Under what conditions is the paracrine loop sufficient to produce aggregation of tumor cells and macrophages?
- How does the size of an aggregate depend on aspects of signalling such as rates of secretion?
- What signalling aspects are most easily changed by drugs to eliminate aggregation?
- How would treatment by drugs affect the process?
- What are the key differences between various cancer cell lines?
- What governs the observed 3 to 1 ratio between motile tumor cells and macrophages?

To address these questions, we introduce two models for interactions and motility of tumor cells and macrophages. The first, described in Section 2.1, is a continuous 1D Eulerian model, amenable to both analysis and simulations. The second (Section 2.2) is a discrete Lagrangian model where individual cells are tracked. In Section 3 we adapt methods from Luca et al. (2003) and Green et al. (2010) to perform a linear stability analysis of the Eulerian model. This leads to conditions for spontaneous aggregation of cells. Results of full simulations of the PDEs and of the discrete model are presented in Section 5. The advantages of this dual approach are that we can use analytical PDE tools to understand parameter dependence (using the continuum model) while preserving our ability to track individual cells and how they move (using the discrete simulation). We summarize our findings in Section 6 and discuss how our models can be useful for designing cancer treatments.

1.1. Paracrine loop and experimental results

Macrophages are a type of white blood cell comprising approximately 5% of the body's white blood cell count. Macrophages originate from monocytes circulating in the bloodstream and are recruited to tumor sites by chemotactic factors such as the colony stimulating factor-1, CSF-1 (Lewis and Pollard, 2006). Up to 50% of the cell mass in breast tumors can be macrophages (Lin et al., 2002). In Table 1 we provide information on cancer cell lines of interest here.

Tumor cells manipulate innate macrophage signalling in order to migrate. The tumor cells secrete CSF-1, which can bind to CSF-1 receptors on macrophages. This activates the macrophages to chemotax towards a CSF-1 gradient and to secrete EGF. The EGF can then bind to receptors on tumor cells, continuing the chain of activation. Activated tumor cells respond by secreting more CSF-1 and chemotacting in the direction of the EGF gradient (Beck et al., 2009; Pu et al., 2007; Goswami et al., 2005; Wyckoff et al., 2004). This process results in a short-ranged chemotactic signalling loop, also called a paracrine loop, see Fig. 1.

The first indication of a macrophages role in tumor cell motility was provided by van Netten et al. (1993). In their experiment, macrophages and tumor cells plated together form multicellular aggregates within 24 h. Wyckoff et al. (2004) conducted *in vivo* experiments in mice to study motility and intravasation (crossing into blood vessels) of tumor cells and macrophages. They used PyMT-induced mammary tumors and a multi-photon microscope to view the process. Tumors were grown for 16–18 weeks after which the anaesthetized mice were viewed under a microscope. Collection needles containing 25 nM EGF were placed inside the tumor. In 4 h, approximately 1000 cells were collected, with 73% tumor cells and 26% macrophages. This ratio of approximately 3:1 tumor cells to macrophages was also observed when MTLn3 cells were grown in rats.

Patsialou et al. (2009) showed that, in addition to the paracrine loop, there can also be a CSF-1/CSF-1R autocrine signalling loop (tumor cells both secrete and respond to CSF-1 gradients). This appears to be the case in some human breast cancer cell lines such as MDA-MB-231, which have CSF-1 receptors in addition to EGF receptors. Results from both *in vivo* (human tumor cells transplanted into mice) and *in vitro* experiments reported in Patsialou et al. (2009) indicate that invasion of the MDA-MB-231 cell line is less dependent on the macrophages. For example, in micro-needles, only 6% of the collected cells were macrophages (compared to 25% in the experiments with MTLn3 and PyMT).

Motivated by van Netten et al. (1993), we will examine conditions necessary for aggregation of tumor cells and macrophages. In view of Wyckoff et al. (2004), we will also explore what

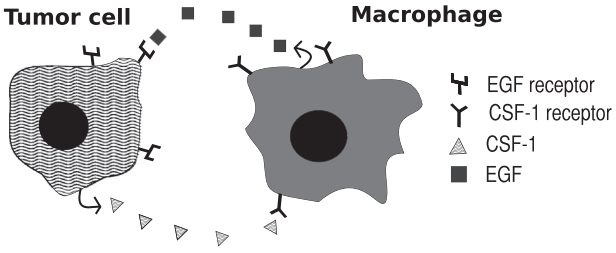

Fig. 1. Macrophages and tumor cells can interact via a short ranged EGF/CSF-1 signaling loop. Tumor cells and macrophages secrete CSF-1 (respectively EGF) and express EGF (respectively CSF-1) receptors. Each cell type responds to the signal from the other type by moving in the direction of the gradient and by secreting its own signal. This paracrine signalling enables tumor cells to migrate with macrophages away from the primary tumor and towards blood vessels or surrounding tissues.

Table 1
Cancer cell lines used in the experiments relevant for this research.

Cell Line	Type	Characteristic	Reference
PyMT	Mouse tumor cell	Highly metastatic (lymph nodes and lungs)	Guy et al. (1992)
MTLn3	Rat tumor cell	Highly invasive and metastatic cell line	Henkels et al. (2011), Wang et al. (2004)
MDA-MB-231	Human tumor cell	Largest known number of EGF receptors per cell also has CSF-1 receptors	Henkels et al. (2011), Price et al. (1999)

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