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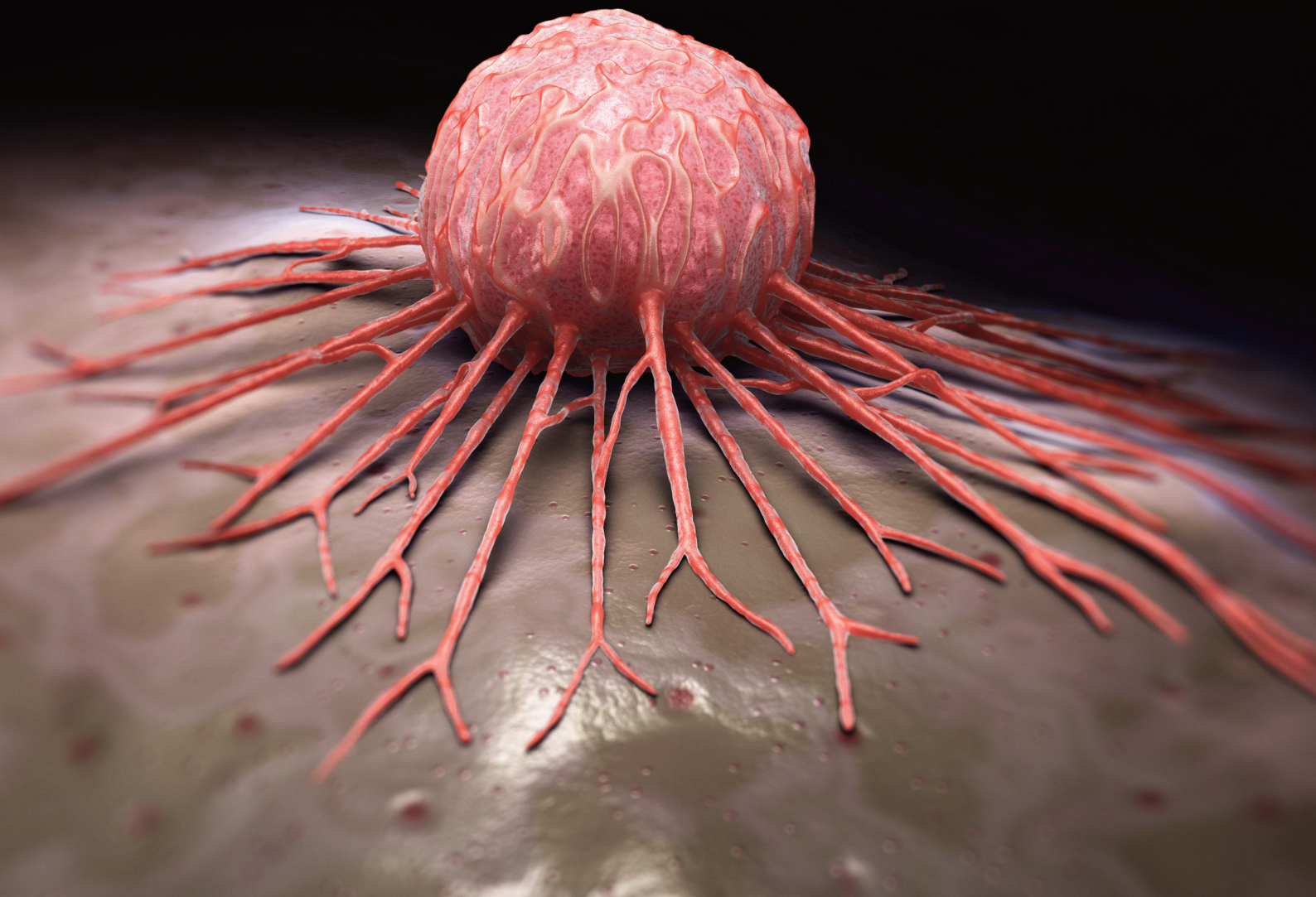
The Clinical Application of Mathematical Pathology



The Clinical Application of Mathematical Pathology

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Mathematical pathology is a research branch of pathology in which mathematics and physical principles are applied to the study of diseases. In the field of cancer research, the objective of mathematical pathology is to model and explain the structural and functional *mechanisms* that control cancer. By understanding these mechanisms, we can provide quantitative predictions of how the tumor will behave. Here, highlighted by our recent work on ductal carcinoma *in situ* (DCIS), we assess and provide the justification for mathematical pathology as a useful way to characterise cancer.

Current clinical practice of DCIS treatment

DCIS is an early form of breast cancer in which the tumor originates within and is confined to the breast duct. While DCIS is not a fatal disease, it often progresses to invasive ductal carcinoma, which accounts for 80% of all breast cancer diagnoses. In many cases, women with DCIS will elect to have breast conservation surgery, in which the tumor and a portion of the surrounding breast are removed. When pathologists examine patient biopsies of breast tissue, they are looking to see whether the neoplastic cells have stayed within the duct (*in situ* disease) or invaded through it. The pathologists are also looking at how aggressive the cells look (i.e., the tumor grade), among other features. Before planning surgery, a team of physicians (radiologists, pathologists, and surgeons) will determine the boundary of the breast tissue affected by DCIS and decide how much tissue needs to be removed. Often, immediately before surgery, a radiologist places a needle or other marker to localise the center of the tumor with the help of X-rays. In the operating room, the surgeon will then measure the amount of tissue to remove around the needle-localised tumor.

Determining the optimal surgical volume for resection thus becomes essential for the control of the tumor and the cosmetic result of the surgery. If too much tissue is removed, the patient could suffer the physical and psychological effects of a large defect that leaves the breast looking uneven; if too little of the affected breast is removed, the patient may need to undergo one or more surgeries to completely remove the affected area of malignant cells. The imperfection of breast conservation surgery is exemplified by the fact that the surgery fails to remove all of the tumor in 38-72% of all cases.

A quantitative approach to breast conservation surgery for DCIS

We sought to address the clinical uncertainty of removing DCIS. We developed a mathematical theory of tumor growth that assumed that the balance of cell proliferation and cell death is a key determinant of tumor size. We also took into account a number of clinical characteristics of DCIS in building the model. For example, younger tumors will have a viable rim characterised by cells in a state of proliferation. More advanced DCIS will be characterised by a rim with more cells in a state of apoptosis (programmed cell death) and a necrotic core of dead cell material that has built up along the central ductal axis as a result of the static pressure delivered by the proliferating cells at the rim of the tumor. By the time DCIS shows up on a mammogram, most patients will have had the disease for at least five months, as this is the time necessary for build-up of micro-calcifications in individual ducts, which are then detectable by mammography. This result indicates that when DCIS is detected, it has usually progressed past the initial fast-growth phase.

The goal of our mathematical pathology work in relation to DCIS is to accurately describe how DCIS develops. By doing so, we would be able to estimate the size of the tumor and aid in therapeutic planning to define the volume of breast tissue which must be surgically removed. An accurate assessment of surgical volume is instrumental in providing physicians and also patients important information before surgery. Through mathematical modeling of cancer, it is possible to describe where a tumor is in its development, and, for DCIS, where the edge of the tumor is located. We designed our approach so that all data being supplied to the mathematical models could be derived from a patient's biopsy and integrated into the clinical workflow.

Mathematical modeling of DCIS

In order to describe the nature and development of specific tumors within a patient, specific values are required for parameters in mathematical equations. In our modeling work on DCIS (Edgerton *et al*., Anal Cell Pathol 2011, PMC3613121), three key parameters are required, and they can all be assessed in pathological analysis of patient biopsies performed *at a single point in time* as a step in between detection through mammography and surgical planning. They are: the **Proliferative Index (PI)**, the **Apoptotic Index (AI)**, and the **diffusion-penetration length**. The fractions of cells in the proliferative and apoptotic states are defined as the ***proliferative index*** and ***apoptotic index***, respectively; the ratio of these two indices is a key parameter in a cell-scale model of tumor growth that forms the starting point for modeling the area of the entire tumor. The **diffusion-penetration length** (or depth) is a measure of how far molecules (e.g., chemotherapy drug, nutrients) diffuse through a medium (e.g., the tumor tissue). Note that the three key parameters are related to tumor development, involve cell proliferation and death, as well as the physical ability of cells to receive nutrients depending on the diffusion properties of the microenvironment, and will provide output useful in determining the tumor margin. Our major purpose with the model was to find out if a specific diagnosis of tumor size is achievable through measurements that are taken from a single biopsy (for quantifying apoptotic/proliferative indices and diffusion penetration length).

We assume that the tumors (of our primary interest here) have passed their initial fast-growth phase and reached a size relatively close to their final volume due to the static pressure balance between tumor cell proliferation and tumor cell death. The tumors in DCIS grow to a point where their growth is arrested due to the

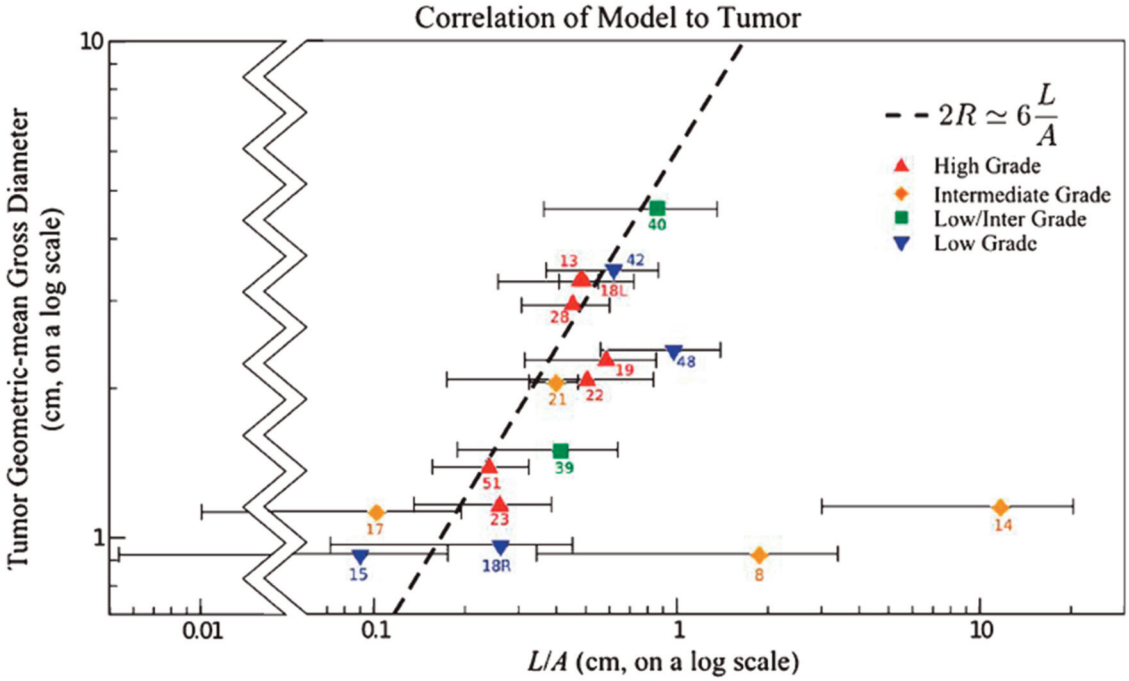

Figure 1. Correlation of tumor size with the death-to-proliferation ratio parameter. Tumor geometric-mean diameters 2*R* (dashed) vs. *L*/*A* predicted by the model compared to the corresponding pathology measurements. In contrast, grades based on histopathology are clearly poor predictors of tumor size. Data were obtained from the 17 excised tumors (symbols, with de-identified case numbers). Reproduced with permission from Edgerton *et al.*, Anal Cell Pathol 34(5):247-63.

lack of viable cells capable of proliferating; at this point nearly all the cells in the core of the duct have undergone lysis (death), with their decayed leftover material contributing to the necrotic core of the duct and accompanying micro-calcifications. Hence, there is a balance between tumor cell proliferation and tumor cell death, and the corresponding ratio of tumor cell proliferation-to-apoptosis, along with the diffusion penetration length. This balance reveals the mechanism behind which the mathematics can model the tumor's growth and predict – when the models are scaled up to the tissue scale – the final geographic area of the tumor, based on one measurement at a single time point.

From a previously developed model (Cristini *et al.*, J Math Biol 2003, 46(3):191-224), we obtain the following analytic solution:

$$A=3.\frac{L}{R}.\left(\frac{1}{\tanh(R/L)}-\frac{L}{R}\right), \quad (1)$$

where A is the patient-specific ratio of cell apoptosis to proliferation rates averaged over the multitude of ducts within the surgical volume, L is the diffusion penetration distance of nutrients, and R is the geometric-mean tumor surgical radius. This equation can be calibrated from the results of immunohistochemistry (IHC), which determines cell proliferation and death. IHC involves staining the cells in the proliferative and apoptotic state, and provides the measurements that drive the proliferative and apoptotic index values. Specifically, in Eq. 1, A and L can be derived from pathology measurements taken on specific patients' tissue (see the original article). When A and L are known, determining the value of R is simply a matter of mathematics. In this way, this equation uses the cell-scale values for calibrating the tissue-scale continuum model predicting surgical volume.

How is mathematical pathology more useful in determining the surgical volume for DCIS

patients than current standard-of-care imaging? Our data show that surgical volumes determined by radiology and tumor grade are largely inaccurate when compared to surgical results. Our study involved examining 17 excised DCIS tumors, and we found that mammography overestimated the tumor size in ten cases and underestimated the tumor size in seven cases. The correlation between mammography, nuclear grade, and the final observed tumor size is poor. However, *the correlation between the sizes observed after surgery and those predicted by the model were close* (see **Fig. 1** for an example). Briefly, model results were very encouraging, with predictions of tumor size and surgical volume being far more accurate when based on patient-specific biomarkers than were the predictions of the same tumor's surgical volume based on mammography.

The implication of the accuracy of our model predictions is that with standard mammogram and pathologic specimens, physicians should be able to accurately predict the surgical volume of DCIS tumors mathematically. This will result in a lower chance of additional required surgeries. The study also confirms that tumor grade or tumor dimensions from mammography are inconsistent with actual tumor volume. Together, this study represents a proof of principle that it is possible to incorporate a mathematical modeling step within current clinical practice to aid in and improve surgical planning by estimating the surgical volume and the outcome of surgery before treatment. Since IHC and morphometric measurements can be performed on patient-specific breast biopsies, the clinical value of this mathematical pathology approach is that the prediction and resulting surgical planning can be tailored for that particular patient. The practice will lead to less subjective analysis of tumors and improved surgical treatment efficacy through individualised treatment design.

Prediction of tumor growth

We then developed an even more detailed model to predict tumor progression starting at the microscopic scale using a lattice-free agent-based modeling (ABM) approach (Macklin *et al.*, J Theor Biol 2012, PMC3322268). This model is the first to account for how cellular calcification influences tumor progression, and is fully constrained to patient-specific clinical data easily obtained from histopathology.

We coupled the cells (i.e., agents) with the microenvironment by introducing field variables for key microenvironmental components (such as oxygen, growth factors, and ECM) that are governed by reaction-diffusion equations. In the model, cells alter the evolution of the environmental variables, and these variables also affect the cells' behavior. We obtained most of the model parameter values from histopathology data analysis using the method described above (Edgerton *et al.*, Anal Cell Pathol 2011, PMC3613121). With parameter values quantified for each individual patient, the model can then be used to simulate the growth dynamics of DCIS. For instance, we used the ABM to predict the quantitative relationship between the mammographic ($\boldsymbol{x}_V$) and pathologic tumor sizes ($\boldsymbol{x}_C$), and obtain:

$$\boldsymbol{x}_V \approx 0.4203 + 1.117\boldsymbol{x}_C \text{ mm.} \qquad (2)$$

We compare the plot of this equation against our simulated DCIS data (blue points; **Fig. 2a**) and a set of published clinical data (red squares; **Fig. 2b**) extracted from (De Roos *et al.*, World J Surg Oncol 2004, 2:4, PMC394346). We find that our model not only correctly predicts a linear correlation between a DCIS tumor's mammographic and pathologic sizes, but also demonstrates an excellent agreement with published clinical data two orders of magnitude larger than our simulation data.

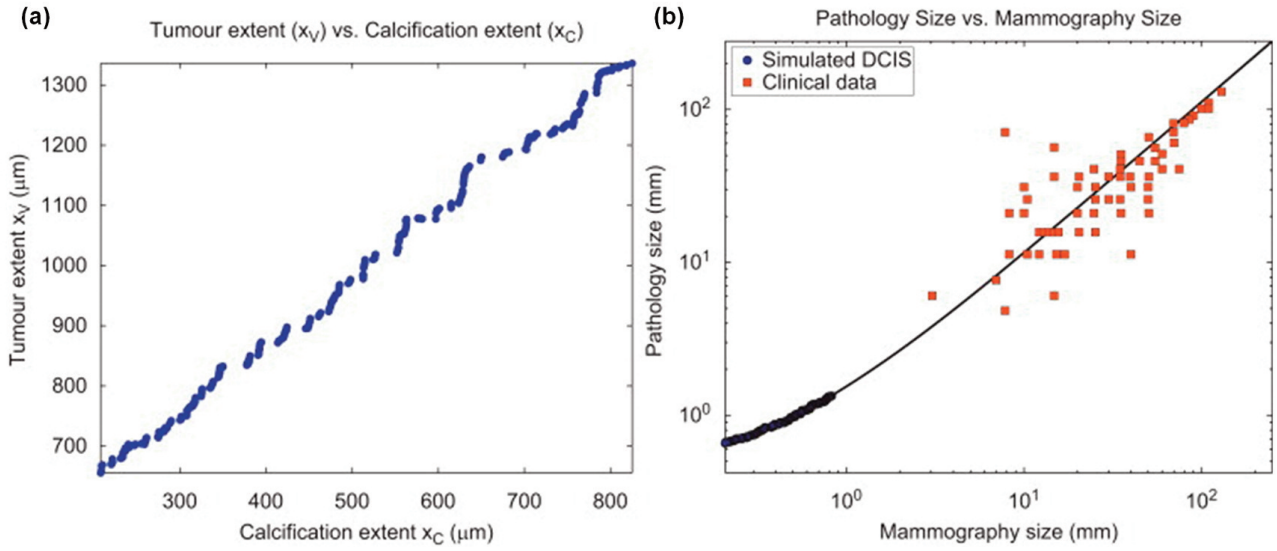

Figure 2. Comparison of mammographic ($\boldsymbol{x}_V$) and pathologic ($\boldsymbol{x}_C$) DCIS sizes. (a) A linear correlation between $\boldsymbol{x}_C$ and the actual pathology-measured $\boldsymbol{x}_V$ is found from our ABM simulation results. (b) A linear least-squares fit to our simulation data (blue circles) fits a clinical dataset (red squares), further demonstrating the predictivity of the ABM model. Reproduced with permission from Macklin *et al.*, J Theor Biol 2012 301:122-40.

These successful quantitative comparisons show that this model may serve as a predictive simulator to create a patient-specific map between the micro-calcification geometry (as observed in mammography) and the actual tumor morphology. The model allows surgeons to more precisely plan DCIS surgical margins while removing less normal tissue. While the mathematics behind the predictions is complex, the model begins with data that is elegant in its simplicity: every portion of data taken from patient pathology can be generated in most cancer centers nationwide – the practice can be replicated just about anywhere cancer is treated, and is the starting point for what drives the models. No extra steps or tests are needed to obtain the necessary parameters that drive the model, making the model easy to apply and convenient to use for physicians.

Clinical implications and future directions

The successes in predicting tumor volume and growth rates based on measurements taken from microscopic pathology illustrate the promise of mathematical modeling of cancer in the development of clinical treatment plans, especially when planning for breast conservation surgery. Integration into clinical trials can help establish this quantitative method as standard practice.

Our mathematical approach also has relevance to cancer therapeutics like chemotherapy. As demonstrated in our other clinically relevant modeling work (Pascal *et al.*, Proc Natl Acad Sci U S A 2013, PMC3761643; Koay *et al.*, J Clin Invest 2014, PMC3973100), macroscopic imaging such as computed tomography (CT)

scans can also be used to derive data to drive models predicting the fraction of tumor killed by chemotherapy in patients with colorectal cancer that metastasised to the liver and in glioblastoma. These will be the foci of future articles.

In the development of our theories and mathematical models, we use data that can be gained and integrated in patient treatment plans in almost any cancer treatment facility. With further research, we hope to show that we can accurately predict clinically relevant outcomes so that these models can assist physicians and patients in making treatment decisions.

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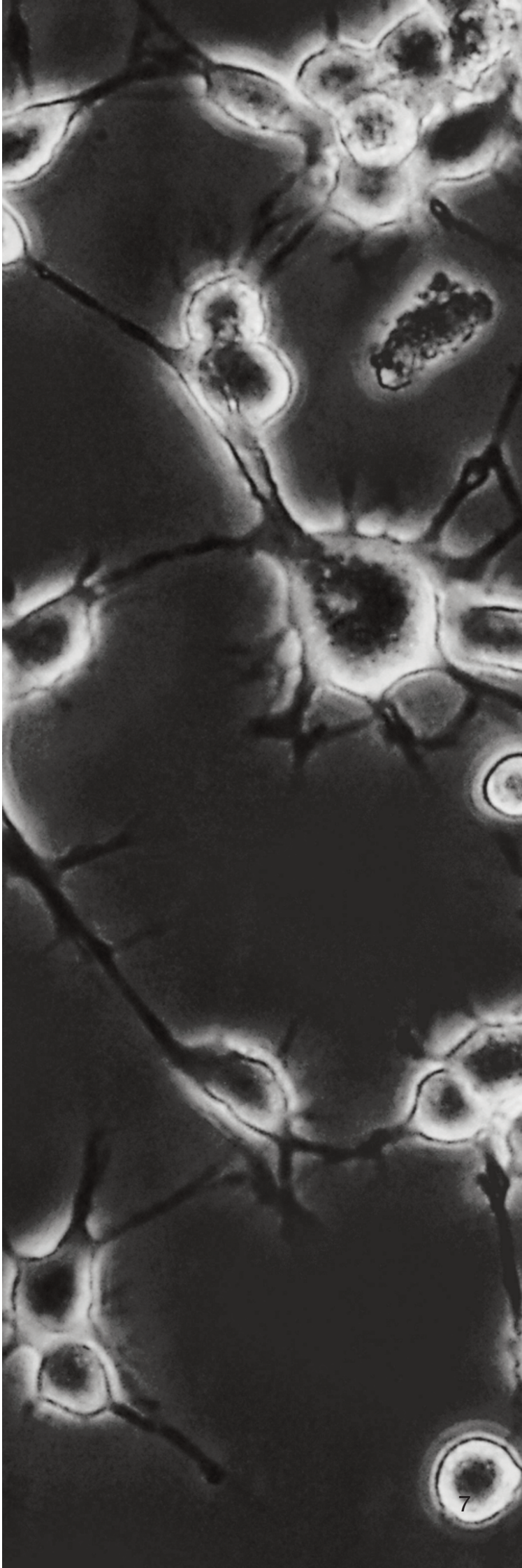
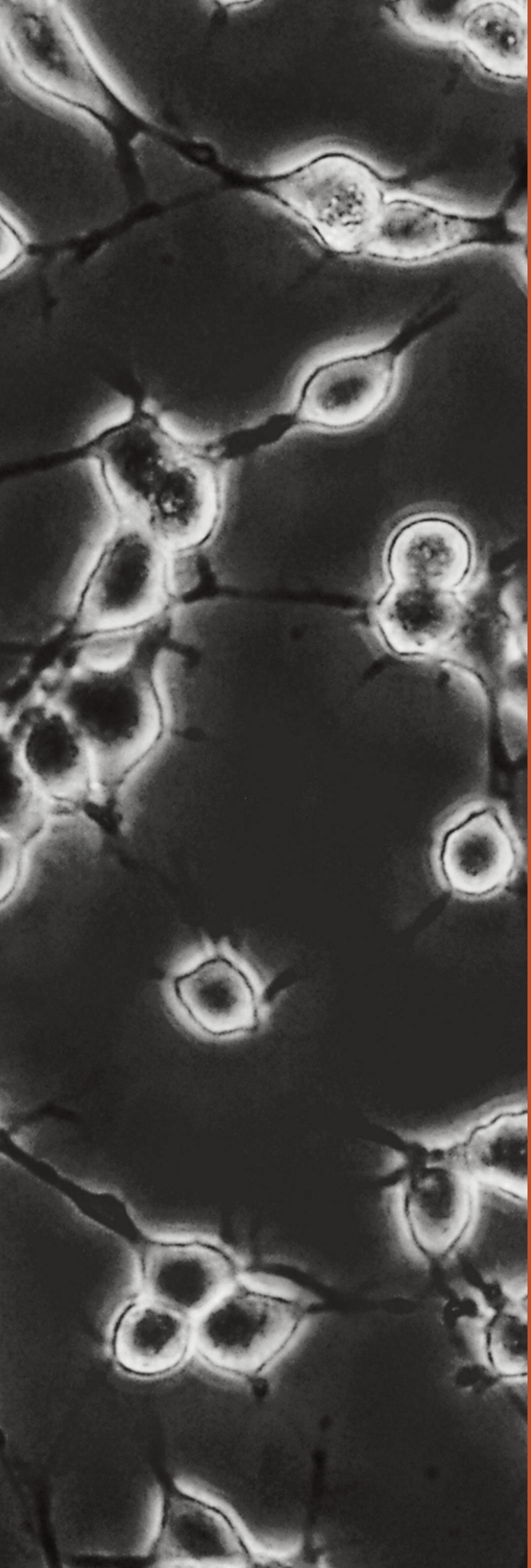
Further reading:

http://physics.cancer.gov/
http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=6673
http://www.internationalinnovation.com/taking-cancer-out-of-the-equation/
http://www.pnas.org/content/110/35/14266.long
http://www.jci.org/articles/view/73455





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