Review

Molecular biology of breast cancer metastasis
The use of mathematical models to determine relapse and to predict response to chemotherapy in breast cancer

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Abstract

Breast cancer mortality rates have shown only modest improvement despite the advent of effective chemotherapeutic agents which have been administered to a large percentage of women with breast cancer. In an effort to improve breast cancer treatment strategies, a variety of mathematical models have been developed that describe the natural history of breast cancer and the effects of treatment on the cancer. These models help researchers to develop, quantify, and test various treatment hypotheses quickly and efficiently. The present review discusses several of these models, with a focus on how they have been used to predict the initiation time of metastatic growth, the effect of operative therapy on the growth of metastases, and the optimal administration strategy for chemotherapy.

Keywords: breast cancer, chemotherapy, mathematical models, metastasis, relapse

Introduction

Breast cancer mortality rates across the entire population in the USA have remained almost unchanged since 1970 [1]. In terms of numbers, it is estimated that 43 300 women died of breast cancer in the USA in 1999 [2]. Those who succumbed to this disease did so as a consequence of metastatic dissemination or the treatment of metastasis. A large percentage of these women were treated with cytotoxic chemotherapy, with drugs that are demonstrated to be effective against breast carcinoma cells both *in vitro* and *in vivo.* Nevertheless, despite being treated with the optimal doses at the optimal schedule, a significant percentage of women will relapse and die. For example, recurrence-free and survival rates at 10 years for women receiving polychemotherapy, for all ages, as estimated in the Oxford Overview, were 44 and 51.3%, respectively [3].

This leads us to ask several questions. First, why are these treatments unable to cure a large percentage of women? Is it the result of cells that are resistant, either kinetically or by means of clonal evolution, to the drugs? Is it a problem of inefficient delivery to the tumor cells or a problem that pertains to the tumor microenvironment? A second question, undoubtedly related to the first set of questions, is why does breast cancer continue to recur up to 20 years after treatment of the primary tumor [4–10].

CMF = cyclophosphamide, methotrexate, fluorouracil.

One discipline that can be helpful in answering the questions posed above is mathematical modeling. It has been observed that trial and error manipulation of cancer treatment can be an inefficient method of understanding and developing treatment strategies [11,12••]. The use of mathematical models can aid researchers by explaining why some strategies fail; by suggesting refinements to current clinical approaches; and, finally, by suggesting alternative treatment strategies based on mathematical models that are derived from both known and hypothesized physiologic phenomena. Furthermore, many variations in the alternative strategies can be tested rapidly *in silico* (on the computer), to determine their effectiveness in a clinical setting. Although modeling strategies cannot replace experimental and clinical results, they can both eliminate some treatment strategies and suggest alternative strategies that may not be apparent just from trial and error manipulation.

Modeling the natural history of breast cancer

Developing a better understanding of the natural history of breast cancer via mathematical models may suggest more effective methods of screening and treatment, and may enable us to answer some of the above questions. A variety of models have been proposed for the natural history of breast cancer. They include models by Speer *et al* [13•], Norton and Simon [14•,15••,16], Spratt *et al* [17,18], and Koscielny *et al* [19••], to list just a few.

The Gompertz model has been the mainstay for models of solid tumors, including breast cancers, for a considerable period of time. The Gompertz model is a modification of exponential growth, with the addition of a decreasing growth rate over time. This decelerated growth causes the cancer to asymptotically approach a limiting size, referred to as its carrying capacity. This limited growth is attributed to several factors, including hypoxia and the lack of nutrients. The origin of this model is a variety of *in vivo* studies in which the Gompertz equation most accurately describes the growth dynamics of the tumor [20]. Using data from Bloom *et al* [21] on the natural history of breast cancer in untreated women admitted to the Middlesex Hospital, London, UK, from 1805 to 1933, Norton and Simon [14•,15••] and Spratt *et al* [17] used this model to describe the data.

Speer *et al* [13•] observed that the subclinical duration of growth given by the original Gompertz growth equation, using a range of parameter values similar to those used by Sullivan and Salmon [22], is too short (approximately 4 months). Also, Heuser *et al* [23] reported that clinical data derived from serial mammograms indicated that nine out of 109 untreated breast cancers measured over a 1-year period showed no growth, and the original Gompertz equation could not account for this observed dormant phase. Thus, they developed a modified Gompertzian model with a stochastic growth rate. This allows for a stepwise growth pattern, with the possibility of dormant phases. In a continued effort to verify this modified model of Gompertz growth with dormant stages and growth spurts, Retsky *et al* [24] reviewed the literature and described a variety of clinical cases in which the traditional exponential or Gompertz model was not consistent with the data.

If the current hypotheses regarding angiogenesis and the development of a tumor microvasculature are correct (see Holmgren *et al* [25] and Folkman [26–28]), then models will need to include some type of dormant phase if they are to accurately account for the complete natural history of the cancer. In fact, Spratt *et al* [17] indicated that, although the original Gompertz model can give a good approximation to clinical tumor growth over the short term, the growth rate of the cancer is more likely to be stochastic over the full history of the cancer. This allows for various growth patterns, including dormancy.

Although initially it may seem that it will make little difference whether either the original Gompertz model or the modified Gompertz model is used to describe the natural history of breast cancer, a quick comparison of the modeling results in the different papers indicates that there are significant differences. For example, using the same data sets Norton [29] (using the original Gompertz growth model) predicted a 2.25-year median preclinical growth phase, whereas Speer *et al* [13•] (using the modified Gompertz growth model) determined it to be approximately 8 years. (Also see the letter by Retsky *et al* [30] in reference to the above-mentioned paper by Norton.)

One of the major impediments to the modeling of breast cancer is our lack of data on its natural history. This makes it difficult to determine whether the growth of a primary breast tumor is a continuous function, or whether it is interrupted by periods of dormancy. Most of the data available comes from serial mammograms. The problem with this is that there are usually only two data points (the estimated size of the cancer when it was diagnosed and the estimated size from a previous mammogram that is reviewed retrospectively) [17,18]. Such a sparse amount of data leads to an indistinguishability problem between the various proposed models (ie biologically different models can describe the same data set equally well). Consider, for example, the exponential equation. Spratt *et al* [17] determined from the mammogram data that the median doubling time is 260 days for breast tumors at detectable levels. Extrapolating back from the approximate detectable size of 10^9 cells, this would indicate that the tumor was initiated about 21 years prior, which seems rather long. In fact, if we consider one of the slower growing breast cancers that they observed, with a doubling time of 7051 days, then the tumor would have been

initiated 578 years prior, which is of course absurd. On the basis of this, we should rule out exponential growth as a viable model of the full natural history of breast cancer.

Modeling breast cancer metastasis

Using the various models of the natural history of breast cancer, researchers have then modeled the initiation time of metastatic growth; the effects of screening on metastases; the effects of surgery on recurrence; and methods of effective adjuvant chemotherapy. These models are used to help address the questions posed in the introduction to this paper.

Estimated initiation time of metastatic growth

The ability to determine the initiation time of metastatic growth would enable us to determine the likelihood of a patient having metastatic recurrence. As should be expected, the predicted initiation time relies significantly on the model of the cancer's natural history. For example, if the model predicts there will be metastatic growth with an early initiation time relative to the diagnosis of the primary (ie there is a high probability that the metastasis has already occurred by the time the primary is diagnosed), then the treatment strategy would be designed differently than if the metastasis were unlikely to have been initiated.

One particular model that considers this question was developed by Koscielny *et al* [19••]. They developed a model to determine the age of metastases at primary tumor diagnosis; the volume of the primary tumor when the metastasis is initiated; the duration of metastatic growth; and the delay between primary diagnosis and appearance of the metastasis. Two main hypotheses play a role in how they developed their model. The first hypothesis is that the metastatic growth rate is proportional to the size of the tumor from which it was derived. The second is that the probability of metastasis is related to the primary tumor doubling time. Using their model, they observed that their data showed that the metastatic initiation occurred at a tumor volume of the primary lesion that was only slightly smaller than that at the time of tumor diagnosis. This is in contrast to the findings of others who used an exponential growth model, with the doubling time of the primary tumor equal to that of the metastatic growth, and predicted that metastatic initiation occurs very early in the development of the primary tumor.

Koscielny *et al* [19••] concluded that the metastatic doubling time is faster than that of the primary (specifically, metastatic growth is about 2.2 times faster than that of the primary); the number of cells needed for metastatic initiation is greater than a single cell; tumor growth is not exponential over the life of the tumor; and growth duration of metastases is approximately 3.8 years, which is much shorter than the previously determined duration of about 17 years (determined using exponential growth and equivalent doubling times for the primary and metastasis). Following these conclusions, they estimated that approximately 30% of patients have metastases that are less than 1 year old, and therefore annual screening would be expected to result in a 30% decrease in the incidence of metastasis. This prediction is in accord with the results of breast cancer screening trials including the Health Insurance Plan trial, the Swedish Centers Combined and Edinburgh Trial ([31] and references therein), which showed a decrease in mortality of approximately 30% for women aged 50–69 years who underwent screening mammography.

Effects of surgery on metastasis

Retsky *et al* [12••] observed a statistically significant bimodal distribution for local and distant, but not contralateral breast cancer relapse using the Milan National Cancer Institute database of 1173 breast cancer patients. This distribution includes a sharp peak of relapse at 18 months and another broad peak at 60 months after surgery. They attributed this bimodal distribution to the effects of surgery on promoting metastatic growth.

To help understand this previously unobserved bimodal recurrence pattern, they developed a stochastic model to attempt to simulate (by Monte Carlo simulations) the clinical results. The model consists of a component to describe the primary tumor growth, based on the model of Speer *et al* [13•] (see the previous section). This is used to describe the release, via a stochastic mechanism, of metastatic cells once the primary tumor is vascularized. The other main component of the model describes metastatic growth and detection, and has three main growth stages. The first stage is a dormant single metastatic cell phase. The second is an avascular stage modeled by Gompertzian growth, with a limiting size of approximately 10^5 cells (or about 0.1–0.5 mm in diameter). The size is limited by the fact that the cells must be nourished by diffusion of nutrients from the existing vasculature. Cells in this stage may remain viable but nongrowing indefinitely. Proangiogenic factors elaborated by the tumor or a downregulation of antiangiogenic factors produced in the stroma, or a combination of both, may result in the induction of a neovasculature that will nourish the metastatic deposit and enable regrowth. This change accounts for entry into the third stage – a vascular stage that is also modeled by Gompertzian growth with a limiting size of approximately 10^{12} cells. The transition between these three phases is considered stochastic.

One of the interesting features of the model is that it allows for an increased progression of metastatic cells to the avascular and the vascular stages immediately after surgery. This was hypothesized to be due to a reduction in levels of tumor angiogenesis factor (produced by the primary tumor) after surgery, allowing angiogenesis to occur at the metastasis and thus allowing rapid growth of

the metastatic lesion. The model without the possibility of stimulation due to the removal of the primary tumor could not produce the bimodal distribution of relapses observed clinically, and only the second peak was observed. However, when the model did account for stimulation of the metastatic cells to stage 2 (avascular) or 3 (vascular) due to the surgical removal of the primary tumor, then the bimodal distribution of relapses similar to that seen in the clinical data was observed. Retsky *et al* attributed the first peak of the bimodal recurrence distribution to metastases in the first two stages before surgery that are then promoted (due to angiogenesis) to the second or third stages.

Adjuvant therapy

Kinetic resistance

One of the early models that attempted to describe effective adjuvant chemotherapy is that of Norton and Simon [14•,15••]. They assumed that all tumor growth, tumor regression, and tumor regrowth is Gompertzian. The initial response of a tumor to chemotherapy is cell death or depopulation. According to Gompertzian kinetics, as the tumor becomes smaller its growth fraction increases, and it regrows at a faster rate. At some point the rate of cell kill may equal the rate of cell repopulation, and the cell population will approach an asymptotic limit. If the asymptotic limit after chemotherapy is always greater than one cell, a cure will never be effected. However, if it is less than one cell, a cure can reasonably be expected. The implication for treatment is that the only way to effect a cure is the absolute eradication of every viable micrometastatic cell. Therefore, Norton and Simon suggested that, when treating micrometastases, high-dose, short-duration treatment (single drug or combination) followed sequentially by a noncross-resistant treatment may be preferable to prolonged duration, low-dose therapy. This treatment strategy challenges the log-kill hypothesis of Skipper *et al* [32•], which suggests that cell kill is proportional to the tumor size.

Clonal resistance

Rather than kinetic resistance, the resistance of cancer cells to chemotherapeutics may be a consequence of clonal selection. The resistance of bacteria to bacteriophages is a random event, as demonstrated by Luria and Delbruck [33••]. A study reported by Law [34], which used an adaptation of the Luria and Delbruck method, concluded that the resistance of the murine leukemia cell line L1210 to A-methopterin, a folic acid antagonist, was also secondary to mutations occurring at random. The Goldie–Coldman model [35] was the first major attempt to place the theory of the evolution of drug resistance by clonal selection into a sound mathematical basis. One of the assumptions of their model is that at each cell division of a nonmutant tumor cell, there exists a fixed nonzero probability that any new daughter cell will be a resistant mutant. By the time a tumor is detected, that is, by the time it reaches 10^9 cells, the Goldie–Coldman model estimates that drug-resistant mutants are present. Also, with an increasing population, the probability that a double mutant is created at random is also increased. Given these assumptions, the optimal way to administer chemotherapy would be to alternate two or more equally effective drugs as rapidly as possible to prevent clonal resistance [36••].

In a similar manner, Panetta [37] defined a model that describes a heterogeneous tumor population and the effects of chemotherapy. The model is used to determine conditions on when treatment should be switched from one drug (or combination of drugs) to a second noncross-resistant drug (or combination of drugs). This condition is related to the ratio of resistant to sensitive cells. The model indicates that the more effective the treatment is, the sooner it will be necessary to switch to the second noncross-resistant treatment.

Tumor dormancy

More recently Retsky and coworkers [12••,38], Demicheli *et al* [39], and Swartzendruber *et al* [40] hypothesized that adjuvant therapy may only benefit those patients who are not cured by local therapy and who would relapse under the first peak of the frequency of the relapse curve (at around 18 months). Smaller tumors with good prognostic factors tend to relapse under the second peak of the frequency of the relapse curve (at around 5 years). Therefore, early detection of tumors could make adjuvant chemotherapy less effective because the metastatic growth has a good chance of being in an avascular dormant stage that is kinetically resistant to adjuvant chemotherapy administered immediately after an operation. Demicheli *et al* [41•] demonstrated the above hypothesis by showing that cyclophosphamide, methotrexate, fluorouracil (CMF) therapy can reduce the risk of metastatic recurrence when compared with surgery alone, but only for patients with recurrences that occur within about the first 3 years. This supports the dormancy hypothesis of Retsky *et al* [12••]. They suggested that either an angiogenesis-inhibiting drug such as angiostatin or the reintroduction of chemotherapy at a later time need to be discussed as alternative treatment strategies.

Another interesting observation made by Demicheli *et al* [41•] is that CMF does not shift the bimodal hazard function to the right (thus just postponing relapse), but rather lowers the peaks. This implies that CMF adjuvant therapy can 'cure' some patients rather than just postpone the recurrence. This idea challenges the view that adjuvant chemotherapy only prolongs survival transiently, and no or very few patients are cured.

Conclusion

How do the various models help us to address the questions posed in the first two paragraphs? What are the

implications of the various cytokinetic models for treatment? To illustrate how mathematical models have been used to design clinical trials, two clinical trials for the adjuvant treatment of breast cancer are briefly discussed.

One of the relatively early clinical trials for women with node-positive breast cancer compared the Norton–Simon model (four courses of adriamycin followed by eight courses of CMF ['sequential']) with the Goldie–Coldman model (two courses of CMF alternated with one course adriamycin for a total of 12 courses ['alternating']) [42]. The 5-year relapse-free survival for the sequential administration arm was 61% and for the alternating administration was 38% ($P = 0.001$). The corresponding figures for 5-year overall survival were 78 and 62%, respectively. The benefit of sequential therapy was observed in all patient subsets.

A clinical trial was designed by the International Breast Cancer Study Group, trial VI, in order to try to examine the possibility that chemotherapy reintroduced at times after the completion of an early postoperative course would be able to treat those cells that have emerged from dormancy [43]. Premenopausal women with node-positive breast cancer ($n = 1554$) were randomly assigned in a 2×2 factorial design to receive the following: CMF for six consecutive courses on months 1–6 (CMF × 6); CMF × 6 plus three single courses of reintroduction CMF on months 9, 12 and 15; CMF for 3 consecutive courses on months 1–3 (CMF × 3); or CMF × 3 plus three single courses of reintroduction CMF given on months 6, 9 and 12.

Patients who were treated with reintroduction chemotherapy had a 5-year disease-free survival rate of $58 \pm 2\%$, compared with $55\% \pm 2\%$ for those who did not receive reintroduction (hazards ratio 0.86, 95% confidence interval 0.73–1.01; $P = 0.07$). The overall survival figures were $76\% \pm 2\%$ and $75\% \pm 2\%$, respectively. Although reintroduction showed a trend toward a therapeutic effect, this difference was not statistically significant. Patients randomized to receive reintroduction had an estimated reduction of relapse of 14% whether the initial chemotherapy consisted of three cycles or six cycles.

Although this study does not definitively support the Retsky–Demicheli model, it could be due to the design of the study. Women who were eligible for the International Breast Cancer Study Group trial VI ranged across the staging spectrum from IIA to IIIA. The benefit of reintroduction may have accrued disproportionately to those women who had primary tumors that were less than or equal to 2.0 cm (T1) with one to three positive nodes, and it may be enlightening to re-examine this subset of patients specifically. Also to be considered when examining the results of the trial are the consequences of prolonged chemotherapy. Compliance with chemotherapy trials is inversely associated with toxicity and duration of treatment. Among the patients assigned to receive reintroduction after six initial courses, 85% started and 75% completed the reintroduction as scheduled in months 9, 12, and 15. This decreased compliance may have diluted the therapeutic effect of the reintroduction regimen in practice. This suggests the possibility that a cytostatic agent, such as an angiogenesis inhibitor, which could be administered chronically with less toxicity, might be better tolerated than an intermittent cytotoxic agent.

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