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A mathematical model of breast cancer development, local treatment and recurrence

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Abstract

Cancer development is a stepwise process through which normal somatic cells acquire mutations which enable them to escape their normal function in the tissue and become self-sufficient in survival. The number of mutations depends on the patient's age, genetic susceptibility and on the exposure of the patient to carcinogens throughout their life. It is believed that in every malignancy four to six crucial similar mutations have to occur on cancer-related genes. These genes are classified as oncogenes and tumour suppressor genes which gain or lose their function respectively, after they have received one mutative hit or both of their alleles have been knocked out. With the acquisition of each of the necessary mutations the transformed cell gains a selective advantage over normal cells, and the mutation will spread throughout the tissue via clonal expansion. We present a simplified model of this mutation and expansion process, in which we assume that the loss of two tumour suppressor genes is sufficient to give rise to a cancer. Our mathematical model of the stepwise development of breast cancer verifies the idea that the normal mutation rate in genes is only sufficient to give rise to a tumour within a clinically observable time if a high number of breast stem cells and tumour suppressor genes exist or genetic instability is involved as a driving force of the mutation pathway. Furthermore our model shows that if a mutation occurred in stem cells pre-puberty, and formed a field of cells with this mutation through clonal formation of the breast, it is most likely that a tumour will arise from within this area. We then apply different treatment strategies, namely surgery and adjuvant external beam radiotherapy and targeted intraoperative radiotherapy and use the model to identify different sources of local recurrence and analyse their prevention.

Introduction

The natural history of breast cancer has been difficult to study. However, in recent years considerable new biological knowledge has accumulated and there are now mathematical models that are attempting to bring together these data. Such mathematical models can also give insights into the mechanisms of existing treatments and help formulate new therapies. Recently simulation models of tumour growth dynamics and response to treatment with irradiation alone have been discussed in the literature (Dionysiou et al., 2004; Zacharaki et al., 2004). In this paper we set out to model breast cancer initiation, progression and recurrence after breast conservative surgery and the effect of radiotherapy on preventing local recurrence.

Mammalian DNA carries genetic information that is stored in chromosomes which are made up by two different chromatids that are joined by a centromere. On each chromatid are so-called alleles with different information from the parent individuals, and the combination of these alleles encodes a gene (figure 1). Oncogenes are genes that are dominant at the cellular level (Michor et al., 2004), which means the mutation of a proto-oncogene allele stimulates the progression to tumour formation – a process known as tumourigenesis. Tumour suppressor genes are recessive genes and the cell does not lose functionality after the inactivation of one of the alleles because the remaining wild-type allele can ensure the functionality and continue suppressing tumourigenesis. When both the alleles are either mutated or non-functional, this suppressive effect is lost which changes the cell's phenotype causing tumours (Iwasa et al., 2005). Therefore, looking at it backwards, allelic loss of one allele of specific chromosomal regions in tumours implies tumour suppressor genes at those loci (Harima et al., 2000; Takebayashi et al., 2001) and is called 'loss of heterozygosity' (LOH). LOH on tumour suppressor genes is important for the initiation or early progression of breast cancer development and is frequently observed in breast cancer and the adjacent tissue

(Dairkee, 1998; Deng et al., 1996; Meng et al., 2004). The segmental distribution of such pre-malignant genetic change in the absence of morphologic change suggests that it could have arisen as a result of clonal expansion of only a few stem cells that mutated before puberty (Tomlinson, 2001; Larson et al., 2002) or pre-pregnancy (Smalley and Ashworth, 2003). Cells carrying LOH in tumour suppressor genes have been shown to have an increased susceptibility to further mutative hits (Li et al., 2002). Therefore, in a model of tumour initiation we could assume that some initial mutations are pre-requisites.

Today, most newly detected breast cancers are of the early stage with a localised tumour due to increased awareness of the disease and widespread use of screening mammography (Apantaku, 2002), resulting in more conservative surgical operations followed by radiotherapy rather than radical treatment (Vaidya et al., 2001). The standard radiotherapy strategy is external beam radiotherapy (EBRT) delivered in 25 fractions of 2 Gy each over a time course of four to six weeks. The time between fractions gives healthy cells enough time to repair radiation-induced damage by homologous recombination or non-homologous end-joining mechanisms (Sancar et al., 2004). The therapeutic index of radiotherapy relies on the fact that cancer cells lack efficient DNA-repair due to down-regulation of, for example, the p53 gene or breast cancer specific BRCA-1 and BRCA-2 genes (Osborne et al., 2004). Also EBRT may miss the target and estimates of such a 'geographical miss' range from 24% to 88% of cases (Vaidya et al., 2005) which could well contribute towards the persistent rate of local recurrence of about a third of the rate without radiotherapy (EBCTCG, 2000).

A remarkable clinicopathological fact is that although about 2/3rd of mastectomy specimens harbour other previously undetected tumours distributed throughout the breast, local recurrence occurs in more than 90% of the cases near the site of the primary tumour (Vaidya et al., 1996 and

2001). Current research focuses on how to obtain tumour control by focussing only on this hazard area and not treating the whole breast. A new strategy, targeted intraoperative radiotherapy (Targit), delivers a single high dose of radiation directed at the tumour bed in the operating theatre while the patient is still anaesthetised. This approach minimises the risk of missing the tumour bed, promises a better cosmetic outcome and reduces the patient's inconvenience (Vaidya et al., 2001). Targit delivers a high dose of radiation to the surrounding area of the applicator surface placed inside the tumour bed, with a rapid dosage fall-off over distance. Near the applicator surface a dosage of about 20 Gy is applied, whereas at a distance of 2.7cm only 1 Gy is left. Figure 2 shows the observed radiation dose as measured by Vaidya et al. (2001) and its approximation with a function with exponential decay ($f(x) = 5e^{1-x^{1.5}}$) as we have previously proposed (Enderling et al., 2006). In this paper we will present a mathematical model of the stepwise development of breast tumours in different pathological scenarios. We shall model the appearance of local recurrence after breast conserving surgery in the absence of radiotherapy and then apply different radiotherapy strategies to model their effectiveness in reducing the risk of local recurrence.

The Mathematical Model

One of the early models of the transformation of normal cells into tumour cells assumes the mutations of an oncogene or a tumour suppressor gene. After the cells have acquired the mutation they become tumour cells which may be modelled as invading the tissue in the form of an advancing wave (Sherrat and Nowak, 1992). Tomlinson (2001) presented a more specific model in which the mutation of two tumour suppressor genes (TSGs) and three oncogenes was necessary to form a tumour cell. We will adapt this approach and for simplicity assume that mutations in two

TSGs are sufficient to give rise to a tumour. These mutations take longer to establish than mutations in oncogenes, which we hypothesise will be mutated simultaneously.

There is an emerging hypothesis that breast cancers could develop from breast stem cells. The existence of breast stem cells has recently been proven by identifying candidate cells which fulfil stem cells criteria, i.e. a long life and a large replicative potential (Smalley and Ashworth, 2003). Estimates of the number of breast stem cells range from 0.2% to 5% of breast tissue cells (Clarke, 2005).

Furthermore breast c*ancer* stem cells with similar properties have been identified in mice (Al-Hajj et al., 2003) and human breast (Clarke, 2005). If we assume that breast stem cells (which already have some cancer cell-like behaviour) develop into breast cancer cells then they have a shorter path to follow in the mutation pathway than normal differentiated cells. So in our model, we let mutations in two TSGs in a breast stem cell create a cancer cell. We will not explicitly model angiogenesis which is necessary for a solid tumour lump to grow beyond a diffusion limited size of a few millimetres in diameter, but argue that one of the crucial mutations may result in the tumour cells releasing angiogenic factors which will force blood vessels to grow towards the tumour and supply it with blood (Anderson and Chaplain, 1998).

The mathematical model we develop in this paper is based on an established partial differential equation model of solid tumour growth and invasion (Anderson et al., 2000; Anderson, 2005; Enderling et al., 2006) which examines how the growing solid tumour invades and interacts with its surrounding environment, i.e. it releases matrix degradative enzymes (MDEs) such as metalloproteinases and urokinase plasminogen activators (Chaplain and Lolas, 2005), which degrade the tissue upon contact to make space for the tumour to grow into (Matrisian, 1992). In

addition to this matrix degradative process through secretion of MDEs, invasion is also facilitated by environmental acidosis caused by tumour cells which causes neighbouring non-tumour cells to die (Gatenby and Gillies, 2004).

In this paper we present an attempt to model the development of cancer cells in a post-pubertal breast from healthy or pre-cancerous cells that we will also use to model the persistence of local recurrence after apparently adequate treatment. The novel extension to the original model of Enderling et al. (2006) consists of 4 differential equations describing the stepwise mutations from a normal breast stem cell to a tumour cell. Spencer et al. (2004) have presented a model in which they investigate various mutation paths to develop a cancer cell. For simplicity, however, we consider a linear mutation pathway and assume that the mutations have to occur in a certain order, i.e. mutations in TSG_2 can only occur after TSG_1 is deactivated. Such a multistage, stepwise carcinogenesis model in the breast has been proposed by Kurose et al. (2001), and similar assumptions were made in models of colorectal cancer development (Michor et al., 2005). For notational convenience we do not distinguish whether the mutation in the paternal allele occurs first followed by the mutation in the maternal allele, or vice versa (i.e., $TSG^{+/-}$ or $TSG^{-/+}$). In the model we take into account that there are two target alleles for the first mutation in a gene compared to only one for the second mutation. The two tumour suppressor genes (TSG) we consider in our model are labelled TSG_1 and TSG_2, and with both alleles un-mutated we refer to them as $TSG_1^{+/+}$ and $TSG_2^{+/+}$. We assume a stepwise mutation pathway as follows:

$$\mathrm{TSG}_1^{+/+}\mathrm{TSG}_2^{+/+} \xrightarrow{p_1} \mathrm{TSG}_1^{+/-}\mathrm{TSG}_2^{+/+} \xrightarrow{p_2} \mathrm{TSG}_1^{-/-}\mathrm{TSG}_2^{+/+} \xrightarrow{p_3} \mathrm{TSG}_1^{-/-}\mathrm{TSG}_2^{+/-} \xrightarrow{p_4} \mathrm{TSG}_1^{-/-}\mathrm{TSG}_2^{-/-}$$

where $TSG_i^{+/-}$ represents a TSG with LOH and $TSG_i^{-/-}$ represents an inactivated TSG. In the final step of the pathway when both of the TSGs are inactivated we assume the cell to be a cancer cell. We have taken the probabilities of the primary and subsequent mutations from the studies by

Tomlinson et al. (1996) and Nowak et al. (2004). Thus, the probability p_1 of mutating one allele of a normal cell is widely assumed to be 2×10^{-7} per gene per cell division. After the first LOH on a TSG, the mutation probability increases by a factor of magnitude 10^1 - 10^2 and hence we assume $p_2 = 10^{-6}$ - 10^{-5}. Once two mutations accumulate within a cell, the probability of further mutations increases to about $p_3 = 10^{-3}$, and the final mutation probability rate p_4 in the model increases as above and is assumed to be about $p_4 = 10^{-2}$. These values are widely used in the literature, and variation of these parameters would only result in a faster or delayed mutation acquisition.

Mutations on genes that are not directly involved in cancer protection but that increase the probability of further mutative hits after being knocked out, i.e. DNA repair genes, may contribute to genetic instability. If we include genetic instability in the model we will assume an increase in the mutation probability rates p_i to be in the range 10^1 - 10^4 (Tomlinson et al., 1996; Spencer et al., 2004).

It is believed that advantageous mutations lead to a selective advantage followed by successive rounds of clonal expansion (Jackson and Loeb, 1998; Boland and Ricciardiello, 1999; Tomlinson, 2001). For normal cells there is an equilibrium of birth and death rate, $a = \textit{birth rate/death rate} = 1$. Mutated cells however, become independent from the tissue, acquire self-sufficiency in growth signals and may evade programmed cell death (Hanahan and Weinberg, 2000). LOH in one TSG may only slightly increase the selective advantage to give $a = 1.01$, but an inactivated TSG will acquire an advantage of $a = 5$, and a tumour cell finally $a = 100$ (Tomlinson, 2001). We will implement these ideas into our model via variations in the proliferation rate.

We denote breast tissue with a fraction of healthy breast stem cells ($TSG_1^{+/+}TSG_2^{+/+}$) by f, cells with LOH in TSG_1 by q, TSG_1 inactivated by r, LOH on TSG_2 by s and tumour cells and the enzymes which they produce by n and m, respectively. Our model of tumourigenesis based on the discussion above therefore consists of six variables and in dimensional form is written as follows:

$$
\begin{aligned}
\frac{df}{dt} &= -\overbrace{\kappa_f\, m\, f}^{degradation} \;-\; \overbrace{\vartheta f}^{f\to TSG_1^{+/-}},\\
\frac{dq}{dt} &= \overbrace{\mu_q\, q\,(A_{\max}-A)}^{proliferation} \;-\; \overbrace{\kappa_q\, n\, q}^{death} \;+\; \overbrace{\vartheta f}^{f\to TSG_1^{+/-}} \;-\; \overbrace{p_2\, q}^{TSG_1^{+/-}\to TSG_1^{-/-}},\\
\frac{dr}{dt} &= \overbrace{\mu_r\, r\,(A_{\max}-A)}^{proliferation} \;-\; \overbrace{\kappa_r\, n\, r}^{death} \;+\; \overbrace{p_2\, q}^{TSG_1^{+/-}\to TSG_1^{-/-}} \;-\; \overbrace{p_3\, r}^{TSG_1^{-/-}\to TSG_2^{+/-}},\\
\frac{ds}{dt} &= \overbrace{\mu_s\, s\,(A_{\max}-A)}^{proliferation} \;-\; \overbrace{\kappa_s\, n\, s}^{death} \;+\; \overbrace{p_3\, r}^{TSG_1^{-/-}\to TSG_2^{+/-}} \;-\; \overbrace{p_4\, s}^{TSG_2^{+/-}\to n},\\
\frac{\partial n}{\partial t} &= \overbrace{\mu_n\, n\,(A_{\max}-A)}^{proliferation} \;+\; \overbrace{D_n\nabla^2 n}^{random\ \ motility} \;-\; \overbrace{\chi\nabla\cdot(n\nabla f)}^{haptotaxis} \;+\; \overbrace{p_4\, s}^{TSG_2^{+/-}\to n},\\
\frac{\partial m}{\partial t} &= \overbrace{D_m\nabla^2 m}^{diffusion} \;+\; \overbrace{\zeta\, n(1-m/m_0)}^{production} \;-\; \overbrace{\omega\, m}^{decay},
\end{aligned}
\qquad (1)
$$

where A_{max} is the carrying capacity of the breast tissue, $A = n + f + q + r + s$ represents the total tissue and cell population including cancer cells, and ϑ is the product of the normal mutation probability p_1 and the proportion of stem cells within the tissue and the number of tumour suppressor genes.

We assume that the tumour grows inside the breast without reaching the outer boundary - the skin. Hence we neglect the breast geometry and assume that the tumour grows in a radially symmetric manner inside a uniformly shaped domain of breast tissue. Therefore our system is considered to hold in a one-dimensional spatial domain Ω (a region of tissue) with appropriate initial conditions for each variable. This domain may be seen as an approximation to a radially-symmetric tumour

geometry within the breast. We assume that tumour cells, and consequently the MDEs, remain within the domain of tissue under consideration and therefore no-flux boundary conditions are imposed on $\delta\Omega$, the boundary of Ω.

In order to solve the system numerically, we first of all non-dimensionalise the equations in the standard way. We rescale distance with an appropriate length scale L (i.e. the size of the breast = 14 cm (Boone et al., 2004)), time with $\tau = 1$ year, tumour cell density with n_0, healthy tissue density with f_0, $TSG_1^{+/-}$ density with q_0, $TSG_1^{-/-}$ density with r_0, $TSG_2^{+/-}$ density with s_0 and MDE concentration with m_0 (where $n_0, f_0, q_0, r_0, s_0, m_0$ are appropriate reference density and concentration variables, and $A_{max} = 1$). Therefore setting

$$\tilde{f} = \frac{f}{f_0}, \tilde{q} = \frac{q}{q_0}, \tilde{r} = \frac{r}{r_0}, \tilde{s} = \frac{s}{s_0}, \tilde{n} = \frac{n}{n_0}, \tilde{m} = \frac{m}{m_0}, \tilde{x} = \frac{x}{L}, \tilde{t} = \frac{t}{\tau}$$

in (1) and dropping the tildes for notational convenience, we obtain the scaled system of equations:

$$\begin{aligned}
\frac{df}{dt} &= -\overbrace{\eta_f\, m\, f}^{degradation} - \overbrace{l_1\, f}^{f \to TSG_1^{+/-}}, \\
\frac{dq}{dt} &= \overbrace{\lambda_q\, q\,(1-A)}^{proliferation} - \overbrace{\eta_q\, n\, q}^{death} + \overbrace{l_1\, f}^{f \to TSG_1^{+/-}} - \overbrace{\rho_2\, q}^{TSG_1^{+/-} \to TSG_1^{-/-}}, \\
\frac{dr}{dt} &= \overbrace{\lambda_r\, r\,(1-A)}^{proliferation} - \overbrace{\eta_r\, n\, r}^{death} + \overbrace{\rho_2\, q}^{TSG_1^{+/-} \to TSG_1^{-/-}} - \overbrace{\rho_3\, r}^{TSG_1^{-/-} \to TSG_2^{+/-}}, \\
\frac{ds}{dt} &= \overbrace{\lambda_s\, s\,(1-A)}^{proliferation} - \overbrace{\eta_s\, n\, s}^{death} + \overbrace{\rho_3\, r}^{TSG_1^{-/-} \to TSG_2^{+/-}} - \overbrace{\rho_4\, s}^{TSG_2^{+/-} \to n}, \\
\frac{\partial n}{\partial t} &= \overbrace{\lambda_n\, n\,(1-A)}^{proliferation} + \overbrace{d_n \nabla^2 n}^{random\ motility} - \overbrace{\gamma \nabla \cdot (n \nabla f)}^{haptotaxis} + \overbrace{\rho_4\, s}^{TSG_2^{+/-} \to n}, \\
\frac{\partial m}{\partial t} &= \overbrace{d_m \nabla^2 m}^{diffusion} + \overbrace{\alpha\, n (1 - m/m_0)}^{production} - \overbrace{\beta\, m}^{decay},
\end{aligned} \tag{2}$$

where $\lambda_i = \tau\mu_i$, $d_n = \tau D_n / L^2$, $\gamma = \tau \chi B / L^2$, $\eta_i = \tau \kappa_i n_0$, $d_m = \tau D_m / L^2$, $\alpha = \tau \zeta n_0 / m_0$, $\beta = \tau \omega$, $l_1 = \tau \vartheta$, $\rho_i = \tau p_i$. We take the value of the cell random motility parameter D_n to be $D_n \sim 10^{-9} cm^2 s^{-1}$ (cf. Bray, 1992). It is known that the MDEs secreted by cancer cells are quickly bound to the matrix and localised near the invading edge i.e. the MDEs have a diffusion rate comparable to the cancer cell random motility. Hence we took $D_m \sim 10^{-9} cm^2 s^{-1}$. The parameter f_0 was taken to be $f_0 \sim 10^{-11} M$ estimated in line with Anderson et al. (2000). We took the haptotactic parameter χ in line with our previous work (Enderling et al. 2006) to be very small in order to reflect early stage breast tumour invasive behaviour which is characterised by very compact, localised spread. Estimates for the kinetic parameters κ, ζ, ω were not available since these are very difficult to obtain experimentally and were therefore taken in line with Anderson et al. (2000).

From $\lambda_n = 0.75$ as estimated in Enderling et al. (2006) and the above presented selective advantages a follows $\lambda_q = a = 7.5 \times 10^{-3}$, $\lambda_r = 1.01 \times a = 7.575 \times 10^{-3}$ and $\lambda_s = 5 \times a = 0.0375$. We define the mutation rates p_i in line with the mutation probabilities stated above $p_1 = 2 \times 10^{-7}$, $p_2 = 5 \times 10^{-6}$, $p_3 = 2 \times 5 \times 10^{-4}$ and $p_4 = 3 \times 10^{-2}$ per cell division.

For the parameters η_i / κ_i representing death of cells due to environmental acidosis caused by the cancer cells, there are no estimates available since these are very difficult to obtain experimentally. We have chosen η_f to be 1 for normal tissue as the MDE which is produced by all cells should easily degrade the tissue and we took smaller values for death parameters to reflect the idea that cell death due to environmental acidosis occurs at a slower rate i.e. $\eta_q = 0.5$, $\eta_r = 0.5$ and $\eta_s = 0.5$, although changes in these parameters will not alter the outcome of the simulations significantly.

We use model (2) to simulate the stepwise development of tumour cells from healthy breast stem cells within the tissue followed by growth and invasion into the tissue. After the tumour has reached a detectable size in our simulations we will then model surgery and apply the two different treatment strategies: (i) external beam radiotherapy (EBRT) and (ii) targeted intraoperative radiotherapy (Targit) to analyse their effectiveness in preventing local recurrence. In line with Enderling et al. (2006) we use the established linear-quadratic (LQ) model to predict the dose-dependent survival fractions S^* for healthy tissue (and the breast stem cells within) f in our model and slightly mutated cells with LOH in one TSG (i.e., $TSG_1^{+/-}$, q in (2)) that have been shown not to have lost their DNA damage repair ability (Nieuwenhuis et al., 2002), and S for the different mutated cell types r, s and n,

$$S^* = \begin{cases} S, & if\ d > 5, \\ S + [(1-S) \times 0.98], & if\ d \le 5 \end{cases} \qquad (3)$$

with

$$S = \exp\left[-N d\alpha \left(1 + \frac{d}{(\alpha/\beta)} \right) \right], \qquad (4)$$

where d is the physical dose delivered per radiation fraction, N is the total number of fractions, α is the coefficient of single-hit DNA double-strand breaks, and β is the coefficient of the combination of two sub-lethal single-strand breaks to form a lethal DNA double-strand break (see Enderling et al., 2006 for further details). We wish to emphasise that in our model as in reality the surgical excision removes the tumour with a rim of normal tissue around it. Hence, radiotherapy is "adjuvant" and meant to act on single stray tumour cells or microscopic foci. Therefore, we have not considered to the effect of hypoxia in the core of the primary tumour (which is already excised).

Assumptions of the model

1) We assume an average fully developed breast size of 14 cm in diameter (Boone et al., 2004) which is the spatial dimension in which we solve our model.

2) Breast tissue is not solid and breast tumours can be felt as a dense lump in the breast. Therefore we assume in our model an average breast tissue density of 0.75, and a maximum tumour density of 1. We assume a tumour detection size of 1.5 cm in diameter above the 0.75 tissue density threshold, which is the average size of early detected breast cancer (Verschraegen et al., 2005).

3) The probabilities for mutations per gene per cell division are known and are as discussed in the previous sections, but the actual number of cancer related tumour suppressor genes remains unknown. Estimates range from several hundred genes that are involved in cancer in general (Jackson and Loeb, 1998; Futreal et al., 2004; Michor et al., 2004) to 30 tumour suppressor genes for lung cancer (Kohno and Yokota, 1999). We assume for our simulations 100 tumour suppressor genes which is in line with the findings of Berardo and Allred (1998) and Devilee et al. (2001).

4) Another assumption in our model is that two *consecutively* inactivated TSGs are neccessary to create a tumour cell. Therefore, the 100 TSGs will be divided in two groups of 50 TSGs each for our probability analysis. One fully mutated TSG from each group is necessary to transform the cell.

5) Estimations of the proportion of stem cells to breast epithelial cells range from 0.2% to 5%, (Clarke, 2005), and we assume 5% of the healthy tissue f in our model is breast stem cells with a proliferation rate of 50 divisions per year (Tomlinson, 2001).

Results of the tumour development model

We now solve the above system of equations (2) numerically using an explicit finite difference approximation scheme in order to simulate (i) the stepwise development of a breast tumour pre-

treatment; (ii) breast cancer surgery, (iii) post-operative treatment with radiotherapy (conventional EBRT and Targit), and finally (iv) the emergence of local recurrence after treatment.

As an initial condition for our model we take a fully developed female breast at a patient age of approximately 20 years. Assuming no mutations have been established pre-puberty, the whole breast tissue is made up of healthy $TSG_1^{+/+}$ $TSG_2^{+/+}$ cells. With the proposed mutation rates per gene per cell division given by p_i and an assumed 5% of the healthy breast tissue as breast stem cells which divide 50 times per year, the probability of developing a tumour within 40 - 60 years is very small. In fact, our simulations have NOT shown the development of a tumour in a fully developed female breast without crucial mutations established pre-puberty. However, if we consider an increase in the mutation rate in the order of $10^1 – 10^4$ due to genetic instability, a tumour develops within clinically observable time, i.e. within 30 years after puberty. This is in line with previous calculations that genetic instability can be a driving force in tumourigenesis if it happens as an early event (Sieber et al, 2003).

Figure 3 shows the results of the step-wise mutation pathway simulations for the development of breast cancer over a period of 30 years assuming 5% of the healthy tissue being breast stem cells and genetic instability $g = 500$, which increases each of the mutation probabilities significantly. To simulate the stepwise development of a tumour we first allow only one mutation ($TSG_1^{+/-}$ $TSG_2^{+/+}$, panel 3a), then two mutations ($TSG_1^{-/-}$ $TSG_2^{+/+}$, panel 3b), three mutations ($TSG_1^{-/-}$ $TSG_2^{+/-}$, panel 3c) and the final fourth mutation ($TSG_1^{-/-}$ $TSG_2^{-/-}$, panel 3d) which is necessary to generate tumour cells. The stepwise increase in selective advantage gives rise to clonal expansion of a mutation which quickly overtakes the populations with less aggressive phenotypes.

If we assume the development of the female breast starts at an age of 10-12 years, we can reasonably expect that some TSGs in breast stem cells have already acquired an LOH mutation ($TSG_1^{+/-}$). We now assume in the model that as few as one of the breast stem cells has acquired this first mutation pre-puberty and thus this mutation has been spread through a certain part of the breast when the breast was formed by clonal expansion via the stem cells (Tomlinson, 2001; Larson et al., 2002). With a high number of already-mutated cells it is possible to run through the complete mutation cascade even without genetic instability as shown in figure 4. We stop the simulation when the tumour diameter reaches a dense (i.e. n>0.75) diameter of 1.5 cm. As the cells with LOH in a TSG, i.e. $TSG_1^{+/-}$ (the light green plots in figure 4) already have one mutation, they are more susceptible to further successive mutative hits and eventually produce a tumour. Our simulations predict that this happens at t = 58.34 years after puberty, representing a patient's age of about 80 years. The mutation model only takes the natural mutation rate into account that occurs when the cell divides. Environmental risk factors increase the probability to acquire mutations and hence can drive the development of a tumour (Jackson and Loeb, 1998). Examples of such environmental factors are ionising radiation, alcohol consumption, or oestrogen/progesterone related risk factors such as early puberty, late menopause, low parity and lack of breast feeding and hormone replacement therapy, which can act as mutagens. We have not considered such external risk factors individually, but in future developments of the model we can include an external mutation factor to scale the results to fit clinical data of the average patient's age of 50 - 60 years.

We now consider the impact of genetic instability as an early event in breast cancer development, which increases in our simulation the mutation probabilities for every mutation by a factor of 500. Assuming this to be the case, then as shown in figure 5, a tumour now develops in a much shorter

time, i.e. 23 years compared to 58 years. Less than 1% of cases of breast cancer are seen at such an early age and those patients that do have been linked with germline mutations in TSGs (Shannon et al., 2003). Thus our model is again consistent with clinical observation. Furthermore, the simulation results also suggests that when the primary tumour is detected, there may be other smaller tumours establishing throughout that breast (bottom panels, figure 5) which could subsequently give rise to new tumours. In the real world, women who develop breast cancer at an early age are at a high risk of developing a local recurrence or a new tumour elsewhere in the breast (Baum et al., 1997; Vaidya 2002; Shannon et al., 2003). Again, our model matches clinical observation. We have shown how mutations lead to pre-tumour progression and finally to tumour growth and invasion. We now investigate and analyse the risk of local recurrence of the tumour after local treatment.

Results of the treatment model and local recurrence analysis

Local treatment is the standard strategy to treat patients with early breast cancer. We present simulations of treatments with (i) conservative surgery alone, (ii) surgery and adjuvant standard EBRT, and (iii) surgery and targeted intraoperative radiotherapy, and then analyse the risk of local recurrence from the different cell types remaining alive in the tumour bed, i.e. normal cells and cells with mutations such as LOH in TSGs. We apply our model (2) to let the tumour grow until it has reached a diameter of 1.5 cm with high density, i.e. above 0.75. We then model the surgical excision of the lump. The tumour margin is set at the boundary of the dominance of tumour cell density over any other cell density in our model, i.e. when $n(x,t) > f(x,t)+q(x,t)+r(x,t)+s(x,t)$. An additional surgical margin of 1cm is taken into account which is based on the theory that tumour cells may escape the main tumour mass, but those remain within a 1cm shell (Ebert and Carruthers,

2003). All densities in this area are set to zero. We then simulate the different radiotherapy strategies and finally apply the model (2) again to analyse the post-treatment dynamics. Figure 6 shows the results of the simulation of surgical removal of a tumour, and we model that some tumour cells may stray beyond the surgical margin (centre panel in figure 6). In our simulations, these will give rise to a local recurrence within a clinically realistic time frame of five to ten years. With surgery alone, a local recurrence occurs in less then 10 years in 30% of cases (EBCTCG, 2000 and 2005) and hence adjuvant radiotherapy is now part of the standard treatment.

We now simulate the effects of different radiotherapy strategies following surgery. Figure 7 (adapted from Enderling et al., 2006) shows the results of the simulation of surgery followed by standard external beam radiotherapy (EBRT), in which 25 fractions of radiation are delivered to the whole breast over a time of four to six weeks. The interval between two consecutive fractions gives the majority of healthy cells enough time to recover from radiation, whereas tumour cells cannot (Osborne et al., 2004; Sancar et al., 2004). Thus, after the application of treatment with EBRT all the tumour cells which remained in the tissue after surgery are destroyed. In addition, a fraction of healthy cells throughout the breast are also destroyed. The application of the initial tumour development model (2) after the treatment shows an increased relapse-free survival time for the patient after adjuvant radiotherapy compared to treatment with surgery alone. In our simulation, new tumours develop about 30 years after treatment in other areas of the breast from healthy tissue by successfully running through the whole mutation pathway again (bottom panels, figure 7).

The results of the simulation of targeted intraoperative radiotherapy (Targit) where a high dose is delivered to the areas of tissue near the applicator surface and there is a rapid dose fall-off elsewhere are shown in figure 8. This radiation strategy, as with EBRT, kills all stray tumour cells

as well. Additionally, the adjacent healthy tissue suffers because of the much higher dose given without any fractionation. However, the distant tissues are spared, which may offer a better cosmetic outcome for the patient. Again the time interval until a new tumour develops is similar to EBRT - about 30 years, which is significantly longer than treatment with surgery alone. We deliberately avoid the term 'local recurrence', as our simulations show that the tumour which arises from the tumour bed formed after surgical excision of the primary lump has run through the full mutation pathway after treatment of the primary disease – and therefore can be said to be a new tumour rather than a true recurrence. In fact, clinical data suggests that 'locally recurrent' tumours may feature mutations which are different from the mutations in the primary tumour and hence could in fact be new primary independent diseases (Schlechter et al., 2004). We note that our simulations predict, in line with clinical data, the development of a tumour at a relatively high patient age. The development of a new primary tumour 30 years later might not be seen, as the patient is likely to have died from age or other diseases.

We have previously discussed the paradox (Enderling et al. 2006) that local control is not complete even if no tumour cells are left behind and that EBRT fails to control the disease in 2/3rds of cases (30% recurrence rate is reduced to 10%). Therefore, there may be other sources of recurrence, such as previously mutated cells which harbour LOH in a TSG in the tumour bed (Enderling et al., 2006).

With the proposed model (2) of the step-wise development of a cancer cell we can now simulate the emergence of a local recurrence from such a field of cells with LOH in a TSG. To avoid the tumour growing beyond the edge of the field of such mutated cells we place an initial tumour on the field's edge (figure 9). We note that the reduced tumour development time of 29.82 years compared to the previously simulated 58.32 years is due to the inclusion of a small tumour nodule

set as an initial condition. Therefore, the tumour growth starts much faster compared to figure 4, where an initial tumour cell has to be developed first.

After the tumour has reached a detectable size we again simulate surgery, and as in the previous simulations there are some stray tumour cells remaining in the tumour bed, but also genetically mutated cells ($TSG_1^{+/-}$). The presence of LOH in morphologically healthy cells in the tissue, adjacent to the primary tumour, will increase their susceptibility to further mutations and eventually give rise to a new tumour (Li et al., 2002). If these cells have no mutations in DNA repair genes, then they will behave similarly to healthy cells and remain relatively unaffected by low-dose fractionated irradiation. However, if there is background genetic instability, i.e. mutations in DNA repair genes, then the low-dose radiation induced mutations will accumulate in the genome and this may speed up the mutation pathway.

We now apply the previously used radiotherapy strategies again to examine their ability to eliminate these new sources of local recurrence. EBRT again kills all stray tumour cells in the tumour bed, but the cells with LOH in a TSG without genetic instability will survive the treatment and compared to previous simulations develop a new tumour in a shorter time interval, i.e. 15-20 years (figure 10).

Targit in contrast delivers such a high dose to the area near the applicator surface, that the majority of the cells with LOH, formed by the same stem cell as the primary tumour, will get eradicated (figure 11), hence providing a longer relapse-free survival time compared to treatment with EBRT. None of the results presented above have been able to reproduce the clinical observation that 10% of cases treated with radiotherapy get recurrence within 5 years of the treatment. We now assume

that the cells with LOH in a TSG, i.e. $TSG_1^{+/-}TSG_2^{+/+}$ have acquired genetic instability at the time of the primary treatment, which is very likely after being subject to mutations for 60 years. For numerical stability we only assume a genetic instability factor of 500, and as shown in figure 12, within 5-8 years after treatment the tumour recurs. Genetic instability increases the mutation probabilities by a factor up to 10^4, and with such an increase in mutation probabilities the re-growth of the tumour can be seen in a much shorter time. However, we were not able to model such a large increase in the mutation probabilities since these high probabilities lead to instability in our numerical scheme used to solve (2). The area of cells with LOH in a TSG is eradicated by the treatment with Targit, and hence the time until a new tumour develops after this treatment is again 25 years as shown above in figure 11. A summary of all presented simulations and time scales until recurrence is given in table 1.

Discussion

We have presented a mathematical model to simulate the stepwise development of breast cancer cells from normal stem cells via mutations in two tumour suppressor genes. Our model predicts that in a fully developed breast which does not harbour any mutated cells post-puberty, the normal mutation rate per gene per cell division might be insufficient to give rise to a fully developed tumour, assuming 100 TSGs and 5% of the healthy tissue being breast stem cells. However, with a variation in the important parameters, i.e. a larger number of cells which have already acquired the first mutation or genetic instability as an early event in tumourigenesis, we have shown that deleterious mutations that lead to clonal expansion can form a tumour within a realistic time frame. The model presented in this paper neglects external factors which are known to increase mutation probabilities (Jackson and Loeb, 1998), and hence our simulation results predicted a patient age of

about 80 years. In a future development of the model we can incorporate an external mutation factor to scale the results to fit clinical data. However, with the current model presented in this paper we can qualitatively compare different treatment strategies as all our simulations are based on the same set of assumptions and parameters.

We have modelled the surgical excision of the tumour and, similar to our previous work (Enderling et al. 2006), used the linear-quadratic model to simulate the death and survival of healthy and mutated cells after the application of different radiotherapy strategies. Our simulations predict that it is very likely that residual stray tumour cells will be eradicated by adjuvant radiotherapy, both EBRT and Targit. We have therefore hypothesised that there may be fields in the breast containing cells with a loss of heterozygosity (LOH) in tumour suppressor genes (TSGs) formed by the clonal development of the breast from a few stem cells that may have become mutated before puberty. All of these cells have an increased probability to give rise to an initial tumour. Our simulations have shown that if a tumour develops at the edge of such a field of cells with LOH, surgery alone may leave some of these cells in the tumour bed and it is these cells which will give rise to a new tumour after running through the remaining mutation pathway. Our model predicts that the low dose of radiation which is delivered in EBRT is not sufficient to kill these cells and hence leaves behind these cells as a highly likely source for a new tumour, i.e. after 15-20 years. If the cells with LOH in a TSG harbour additional genetic instability, which increases the mutation probability by a factor of 500 in our model, then the recurrent tumour can be observed within 5-8 years of treatment. Genetic instability has been discussed in the literature and is known to increase the mutation probability by a factor of up to 10^4 which would result in a recurrent tumour much sooner than it is seen in the clinic, i.e. in the first 2-4 years. In contrast to conventional radiotherapy, the new targeted single-dose irradiation with Targit delivers a high dose of radiation to the tissue which

surrounded the original tumour, and hence the cells with similar mutations to the tumour are also most likely to be eradicated. We have shown that Targit might be the better treatment strategy in terms of preventing true local recurrence. This, however, does not prevent the development of a new independent tumour at the location of the original tumour or elsewhere in the breast. In fact, if we let our simulations run for long enough, even after the treatment of the primary tumour with Targit, there is a possibility of developing a new tumour (but not a local recurrence of the primary tumour).

In this paper we have focussed on primary tumourigenesis in a post-pubertal breast. In future work we will study the formation of the breast itself in order to model field effects in the breast in an anatomically correct manner. Furthermore, we plan to investigate the effect of radiation-induced mutations on the development of secondary malignancies (Durante et al., 1996; Obedian et al., 2000; Lorimore et al., 2001; Goldberg, 2003; Turesson et al., 2003) to compare the disadvantages due to the side effects of the radiation strategies described here in this paper. To account for the stochastic measures involved in this process, as well as in the development of the tumour, we plan to develop a discrete individual cell based model in the future.

Conclusions

The model discussed here is a first attempt at modelling complex biological phenomena of which only little is known to date. Only recently have breast stem cell candidates been identified, but the research is still far from identifying all and exactly how they are involved in the development of the breast. Also the identification of tumour suppressor genes has been a major task in recent years with much research still to be done. The model framework presented here has predicted that given

established mutation rates, there must exist many susceptible genes and stem cells to make breast cancer the most common malignant disease for women today (Weigelt et al., 2005). Breast cancer will affect 12% of all women (van Diest et al., 2004), and hence motivates further research in this area. Our model of treatment has shown that different radiotherapy strategies, i.e. standard fractionated external beam radiotherapy (EBRT) and single dose targeted intraoperative radiotherapy (Targit) are most likely to eradicate stray tumour cells which might have been left behind in the tissue after surgery. However, a high dose of radiation directed at the tumour bed, as delivered by Targit, may also eradicate sister clones of the cell which gave rise to the primary tumour. We hope that the work presented in this paper will stimulate experimental research groups to further investigate the field effect of mutated cells (Deng et al., 1996) as it is these cells that are likely to cause treatment failure.

The model presented in this paper focuses only on one aspect of the complex process of tumourigenesis. This is of course an oversimplification, but despite this the simulation results from our model can successfully reproduce experimental and clinical data.

The treatment strategies discussed in this paper aim to achieve local tumour control. However, with tumour progression being driven by random events, neither of the techniques can prevent the development of a new local tumour or a distant tumour, and we have therefore refrained from calling these new diseases local recurrence. Our next task is to refine and expand the model as discussed to focus the development towards a simulation-supported interactive treatment planning tool.

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Figure 1. Schematic representation of a chromosome, with different alleles on the two chromatids encoding one gene.

Figure 2. The radiation dose which is delivered by Targit as measured by Vaidya et al. (2001) and its approximation by a function f(x) as proposed by Enderling et al. (2006).

Figure 3. Mutation pathway analysis using tissue and cell densities after 30 simulated years. When only the first mutation from breast stem cells within the healthy tissue (*f*, dark green) to cells with LOH in one TSG (*q*, light green) is allowed (a), normal cells get converted into $TSG_1^{+/-}$. With a second mutation enabled (b) which knocks out the second allele of the first $TSG_1^{-/-}$ (*r*, cyan), the obtained selective advantage of the new cells will quickly overgrow the $TSG_1^{+/-}$ cells (light green). A further mutation (c) to the second $TSG_2^{+/-}$ (*s*, orange) is achieved very quickly with a high selective advantage so that compared to (b) the developed $TSG_1^{-/-}$ region is no longer be formed. The introduction of the final mutation (d) which creates tumour cells (*n*, red) overgrows all other previously established clonally formed areas as their selective advantage is much higher. After 20 years, the tumour has progressed so much that only a fraction of the initial healthy tissue is left. The probability for mutations to occur is equal throughout the domain and hence no spatial variation.

Figure 4. Results of a simulation of the evolution of a tumour (red) in a breast with healthy cells (dark green) and cells with an LOH predisposition (light green). Cells in this area are most likely to receive further mutative hits and eventually give rise to the next mutation and so on which leads to the development of a tumour in $t = 58.34$ years after the breast has been fully

developed. The thin black line shows the concentration of matrix degradative enzymes, which are released by the tumour and destroy the matrix upon contact.

Figure 5. Development of a breast tumour within an area of cells with LOH in TSG including genetic instability as an early event of tumourigenesis. A tumour (red) develops and grows up to a detectable size much faster than without genetic instability being involved (23 years compared 58 years). At the time when the first tumour has developed, there are other tumour cells already established all over the domain, i.e. breast, which may quickly grow to form tumours of detectable size. The thin black line shows the concentration of matrix degradative enzymes, which are released by the tumour and destroy the matrix upon contact.

Figure 6. Surgery after the development of a tumour from within a field of LOH on a TSG. The left panel shows the result of the stepwise development, growth and invasion of a tumour (red) developed from a field of cells harbouring LOH in a TSG, i.e. $TSG^{+/-}$ (light green) into a domain of healthy cells (dark green). The pre-treatment development process is shown in figure 4. After the tumour reaches a detectable size at t=58.34 years, it is excised with an additional 1cm margin (centre panel). The red areas showing at the bottom of the middle panel show tumour cells that have been left behind after surgery, and it is these areas from which local recurrence arises within 5-10 years after surgery (right panel). The thin black line shows the concentration of matrix degradative enzymes, which are released by the tumour and destroy the matrix upon contact.

Figure 7. Surgery and adjuvant radiation with external beam radiotherapy (EBRT).

The initial tumour growth and surgery is shown in figure 4 and 6. The top left panel shows the situation after surgery with a few tumour cells (red) left behind in the healthy tissue (dark green). The top middle panel shows the first irradiation fraction with a uniform dosage distribution of 2 Gy all over the domain (thick black line). The panels in the middle row show the result after the third, tenth and twenty-fifth fraction respectively. After the 25^{th} fractions no tumour cells remain in the tissue and only a small fraction of healthy tissue throughout the breast has died as a result of the treatment. The time interval until a new tumour has developed in the breast (bottom row, 30-35 years after treatment) is significantly longer compared to treatment with surgery alone (5 years after treatment).

Figure 8. Simulation of surgery and adjuvant radiotherapy with Targeted intraoperative radiotherapy (Targit). The initial development of the tumour and its treatment with surgery alone is shown in figure 4 and 6. After the surgical excision of the primary lump (top left panel) a single high dose (the thick black line in the top middle panel shows the dose distribution) is applied with Targit. The stray tumour cells (red) are eradicated, and again healthy tissue (dark green) also suffers from the treatment. The top right panel shows the healthy tissue which is left after the treatment. In contrast to EBRT the damage to healthy cells is very localised inside the breast, which promises a better cosmetic outcome. The time interval until a new tumour has developed in the breast (30-35 years after primary treatment, bottom row panels) is similar to recurrence after EBRT and significantly longer compared to treatment with surgery alone.

Figure 9. Surgery after the development of a tumour from the edge of a field of LOH on a TSG. The panels show the stepwise development, growth and invasion of a tumour (red) which has initially been placed on the edge of a filed of cells with LOH in a TSG, i.e. $TSG^{+/-}$ (light green, top left panel) and then developed into a domain of healthy cells (dark green). After the tumour reaches a detectable size at t=29 years after puberty, it is excised with an additional 1cm margin (bottom right panel). The red areas showing tumour cells at the bottom of the panel which have been left behind after surgery as well as a field of light green cells with LOH in a TSG.

Figure 10. Surgery and adjuvant radiotherapy with external beam radiotherapy (EBRT) and a field of cells with LOH in a TSG in the tumour bed. The initial tumour growth and surgery is shown in figure 9. The top left panel shows the situation after surgery, with some tumour cells (red) and cells with LOH in a TSG, i.e. $TSG^{+/-}$ (light green) left behind in the healthy tissue (dark green). The middle panel in the top row shows the first fraction with a uniform dosage distribution of 2 Gy all over the breast (thick black line). The top right panel shows the result after the delivery of the first fraction. The panels in the middle row show the results after the fifth, tenth and twenty-fifth fraction. All tumour cells are eradicated, but some of the $TSG^{+/-}$ cells survive this treatment, and it is these cells which ultimately form a new tumour (i.e. 15-20 years, bottom row).

Figure 11. Simulation of surgery and adjuvant radiotherapy with targeted intraoperative radiotherapy (Targit) and a field of cells with LOH in a TSG. The initial tumour growth is shown in figure 9. The top left panel shows the result after surgery, with a few stray tumour cells (red) left behind in the tumour bed as well as a field of cells with LOH on a TSG, i.e. $TSG^{+/-}$ (light green). After the surgical excision of the primary lump a single high dose of radiation (the thick black line in the top middle panel shows the dose distribution) is applied with Targit. After radiation treatment (top right panel) all tumour cells and the field of cells with LOH are eradicated because of their close location to the applicator. Distant healthy tissue (dark green), however, survives the treatment. The panels in the bottom row show the simulation and analysis of local recurrence. The first 20 years after primary treatment are relapse free.

Figure 12. Recurrence after EBRT from cells with LOH and genetic instability. The simulation of the delivery of EBRT is shown in figure 10. With genetic instability in cells with LOH in a TSG, i.e. $TSG_1^{+/-}$ a new tumour develops within 5-10 years of the surgery.

Table 1. Time scales until recurrence with the different treatment strategies considering different tumour bed properties.

	surgery	surgery + EBRT	surgery + Targit
no cells with LOH in tumour bed	5 years	30-35 years	30-35 years
cells with LOH in tumour bed		15-20 years	25-30 years
cells with LOH + GI in tumour bed		5-7 years	25-30 years

LOH – loss of heterozygosity, GI – genetic instability, EBRT – external beam radiotherapy, Targit – targeted intraoperative radiotherapy

Fig. 1

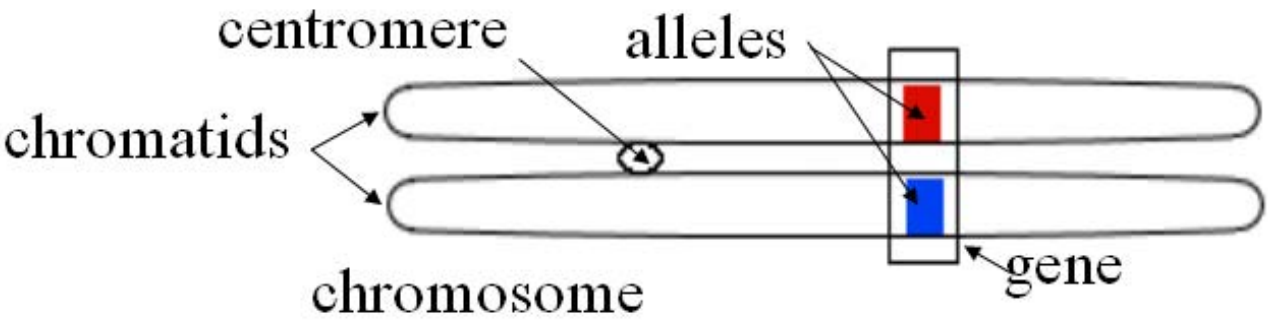

Fig. 2

distance cm	dose Gy	$5e^{1-x^{1.5}}$ Gy
0.1	15	13.17
0.2	12.5	12.43
0.5	8.75	9.54
1.0	5.0	5.0
2.7	1.0	0.16

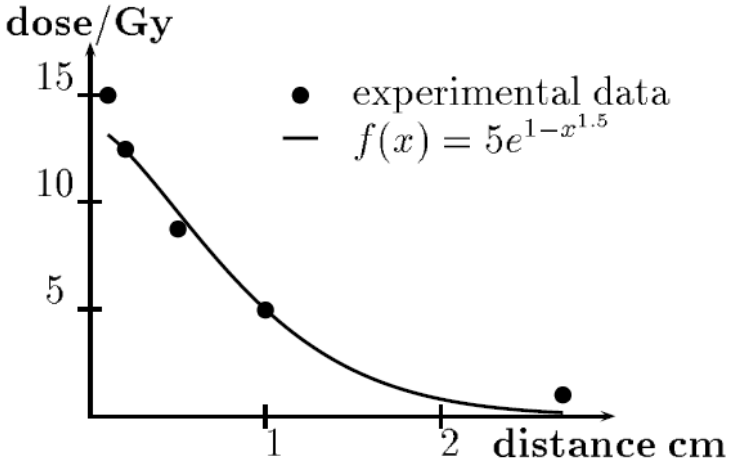

Fig. 3

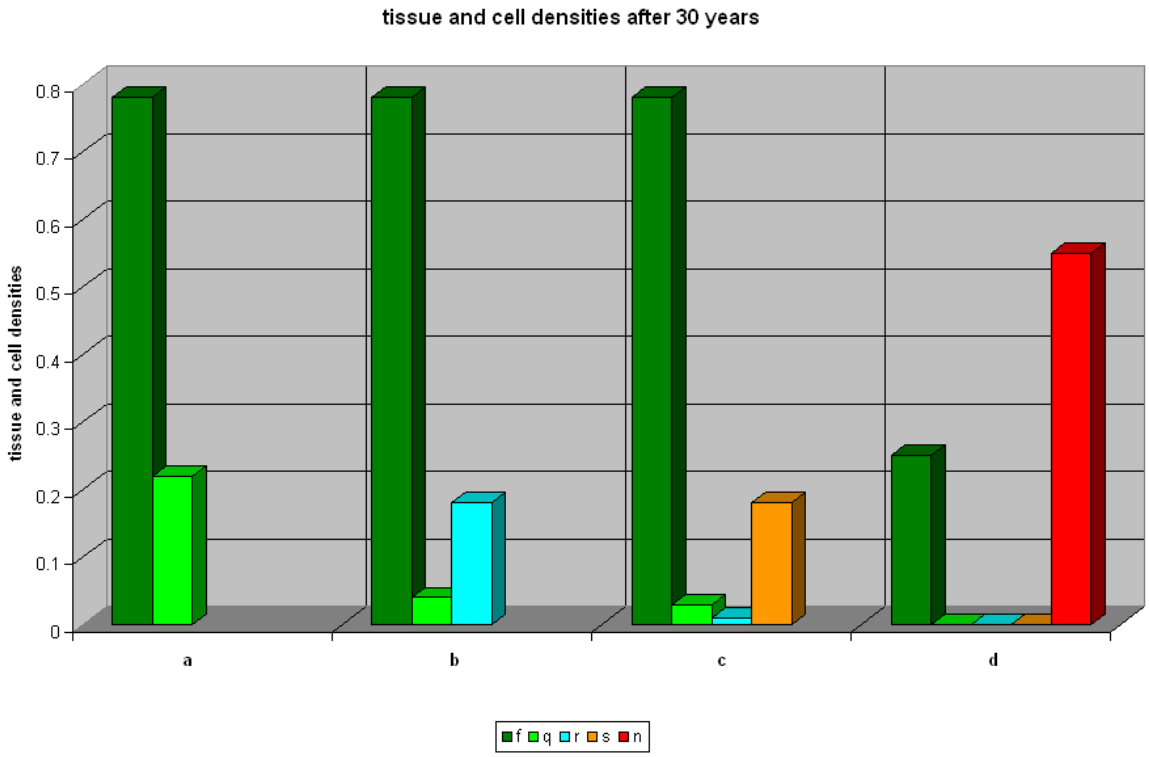

Fig. 4

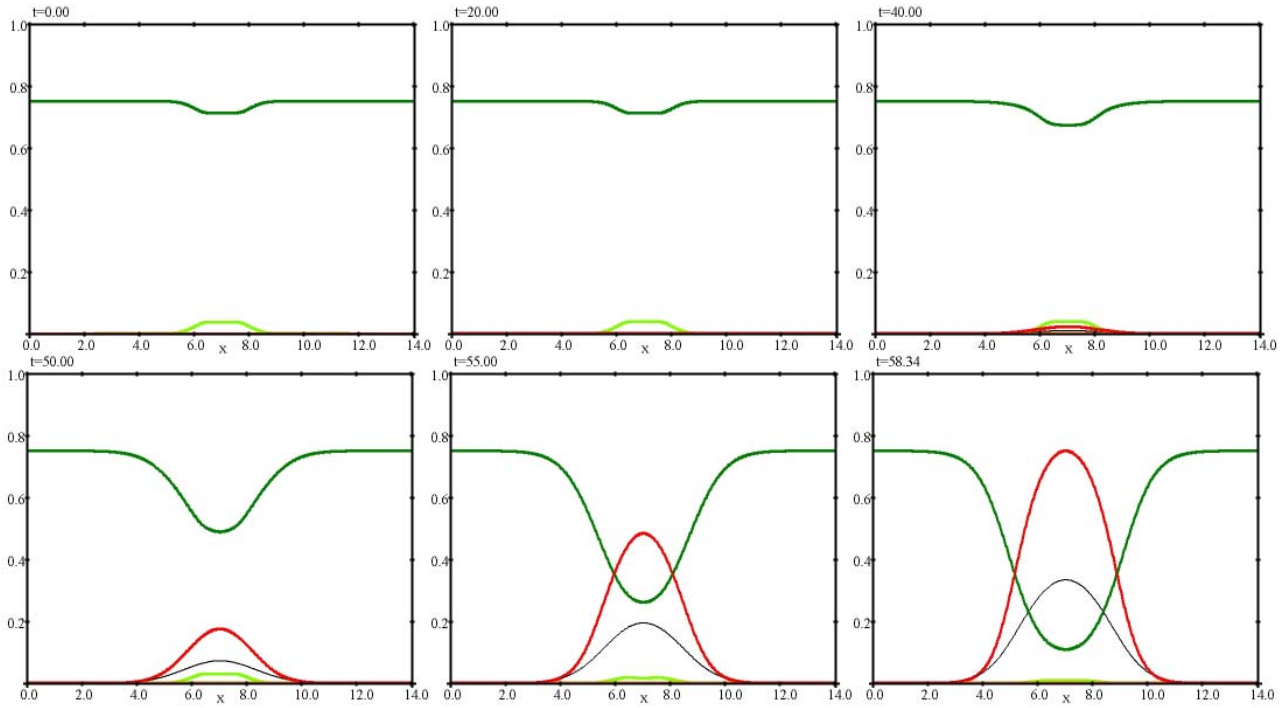

Fig. 5

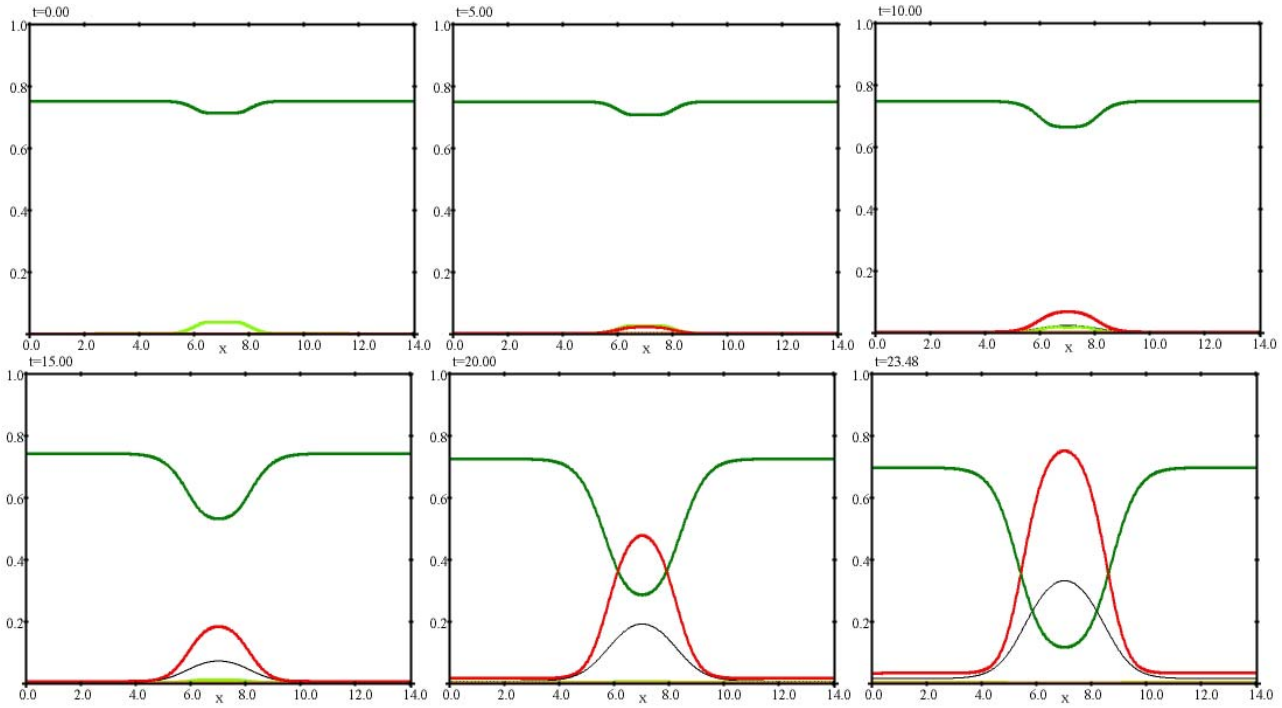

Fig. 6

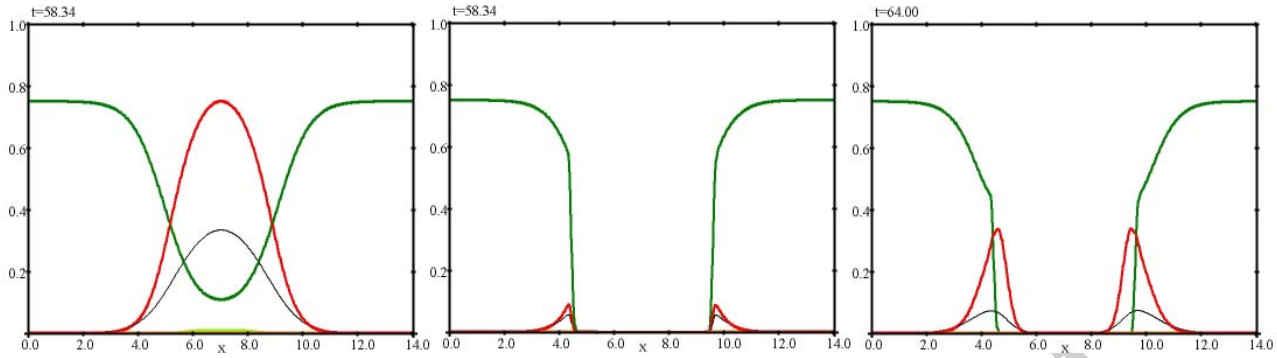

Fig. 7

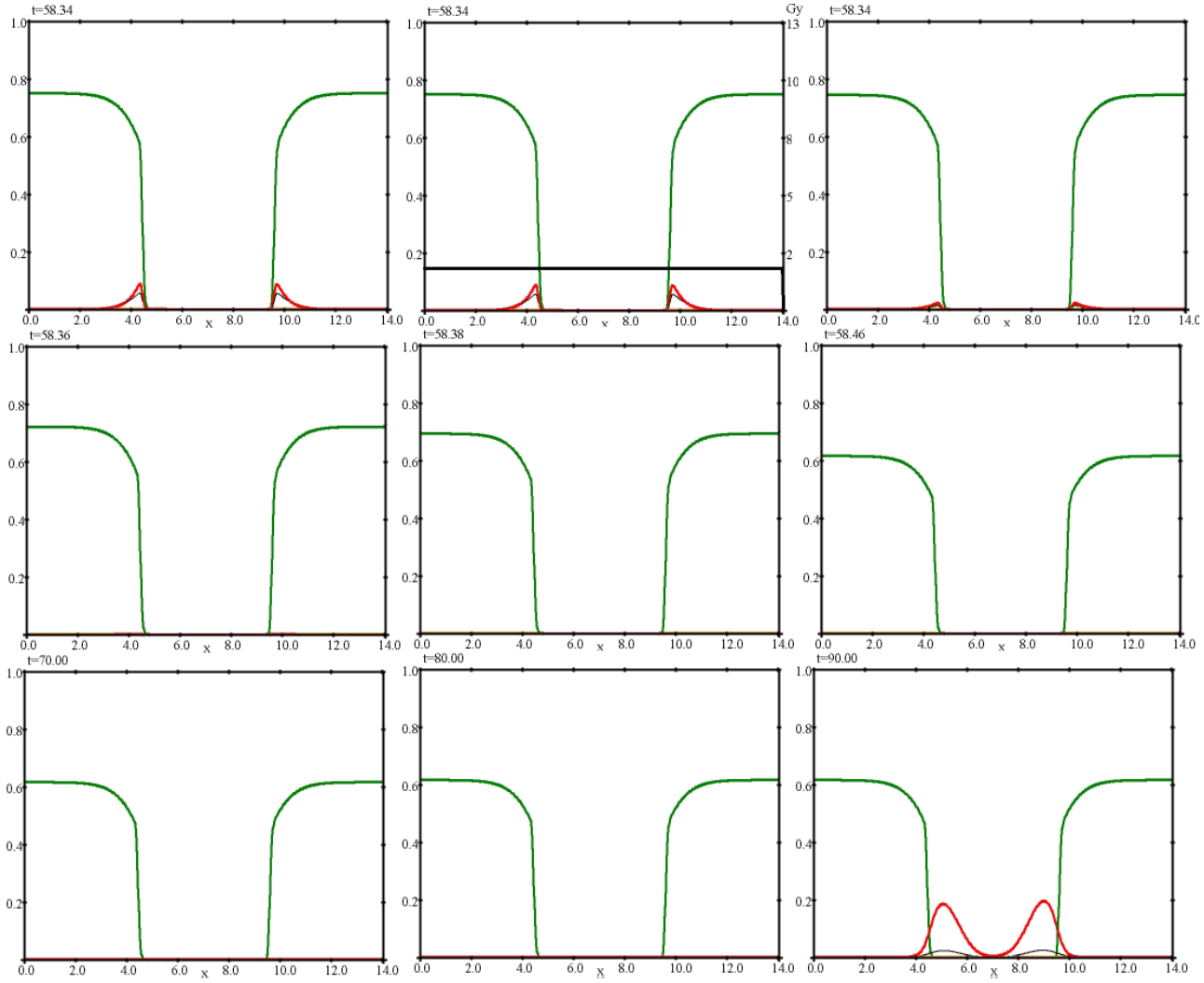

Fig. 8

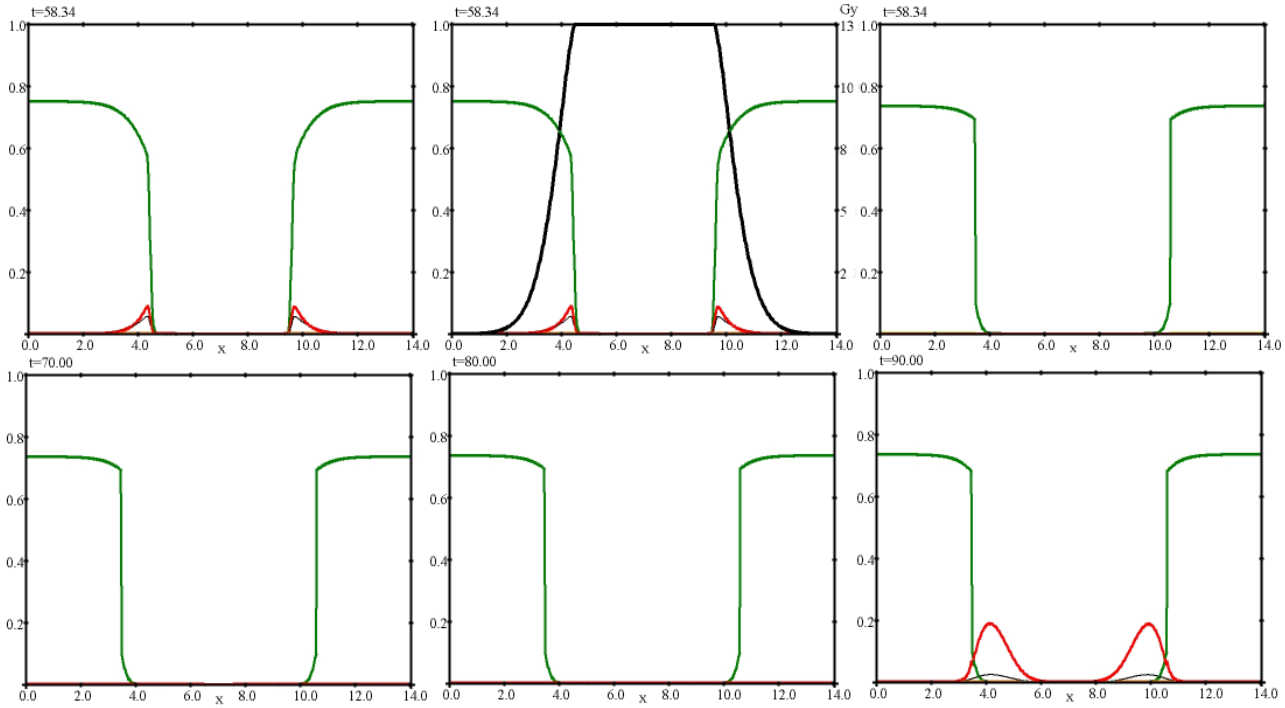

Fig. 9

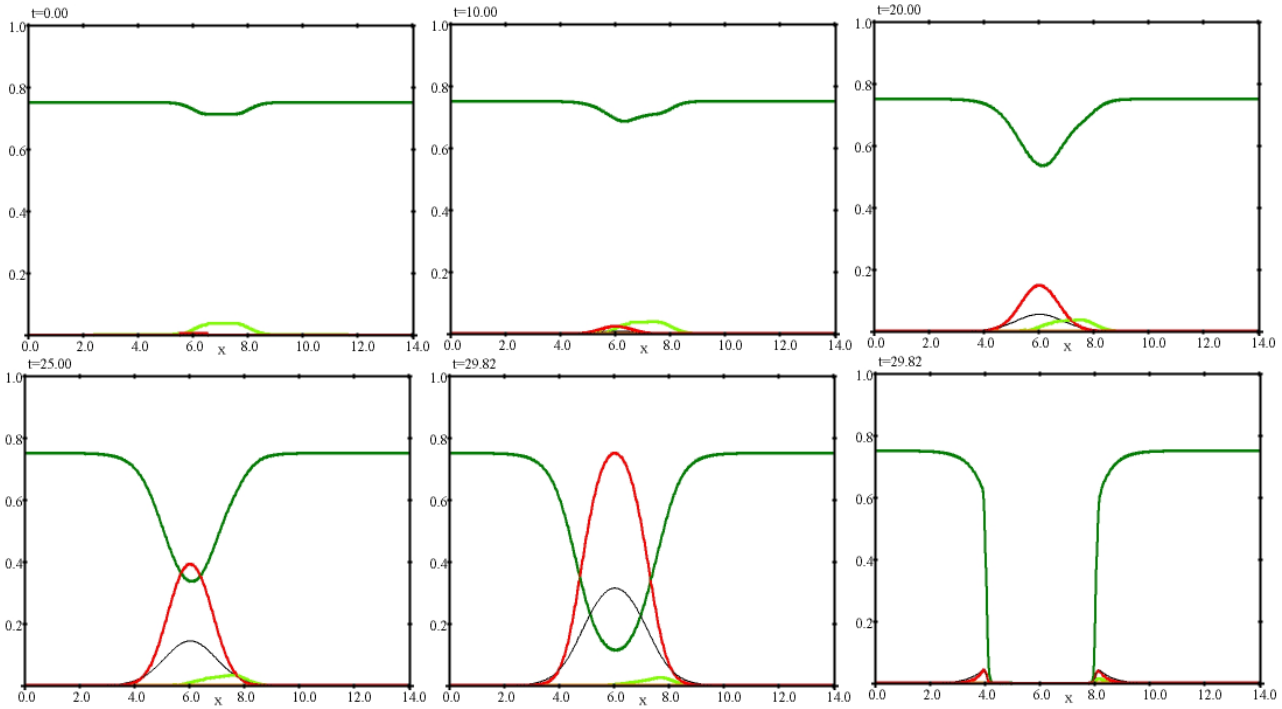

Fig. 10

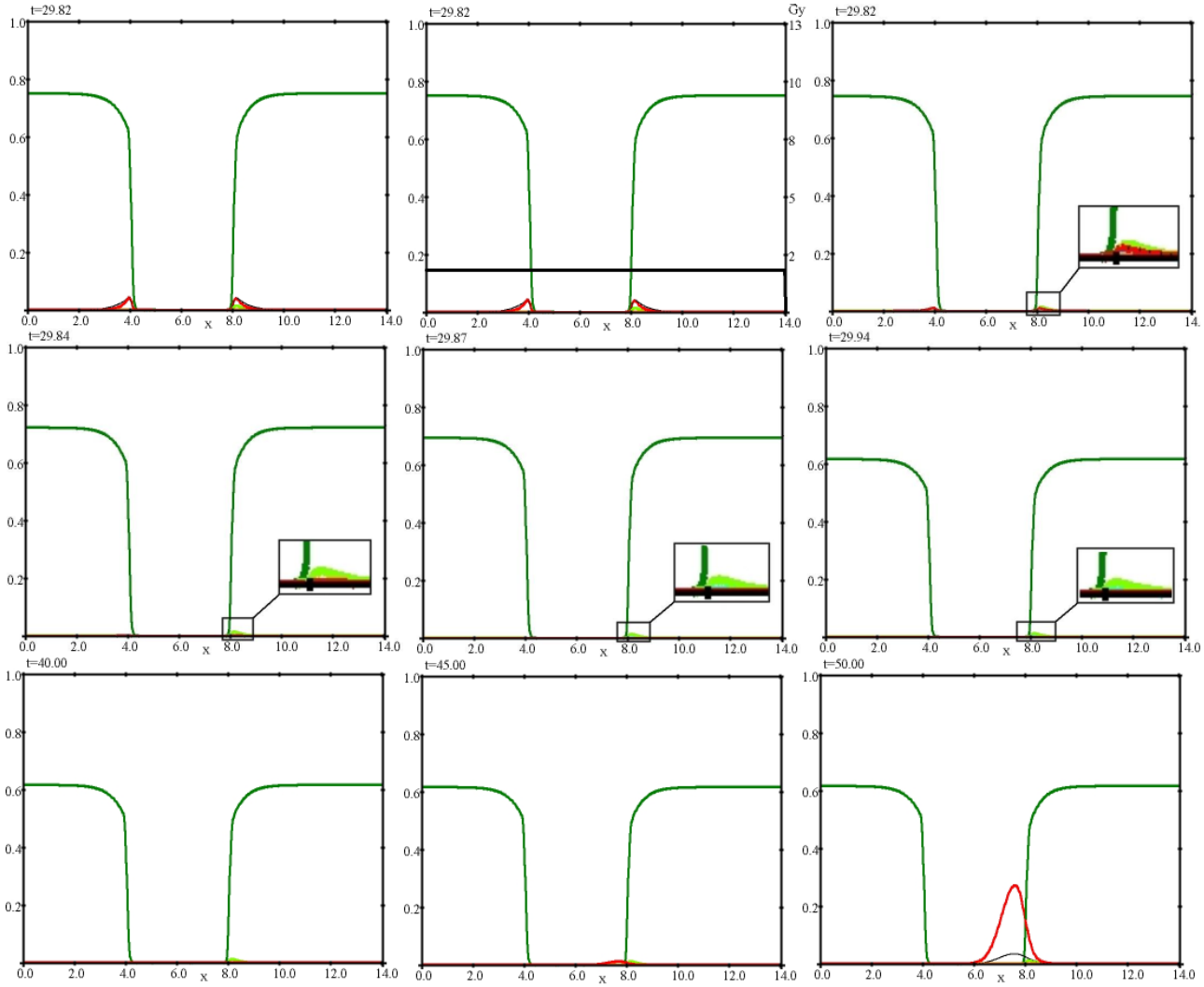

Fig. 11

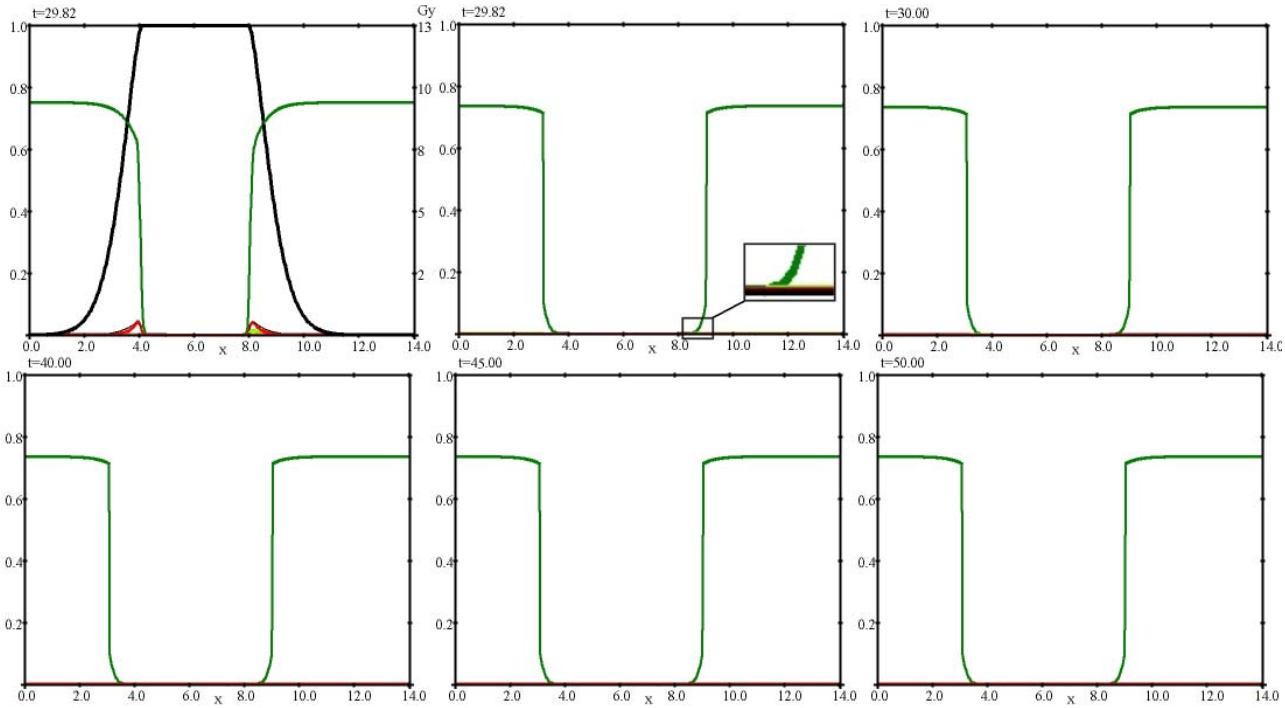

Fig. 12