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European Journal of Cancer 37 (2001) 1545–1553

European Journal of Cancer

www.ejconline.com

The relative importance of proliferation and cell death in breast cancer growth and response to tamoxifen

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Received 14 September 2000; received in revised form 4 April 2001; accepted 1 May 2001

Abstract

Changes in tumour volume depend on the relative balance between cell proliferation and cell loss. However, these processes are not independent, and it remains unclear which is more important in tumour progression and regression. For example, the anti-oestrogen tamoxifen, a mainstay in the therapy of breast cancer, has both antiproliferative and pro-apoptotic actions, and their relative importance in clinical efficacy is unknown. Thus, using a model system based on oestrogen receptor (ER)-positive ZR-75-1 breast cancer xenografts, both increased apoptosis and reduced proliferation have been previously shown to occur within 7 days of tamoxifen therapy (Cameron DA, Ritchie AA, Langdon S, Anderson TJ, Miller WR. Tamoxifen induced apoptosis in ZR-75 breast cancer xenografts antedates tumour regression. *Breast Cancer Res Treat* 1997, **45**, 99–107). In the present study, Gompertzian growth curves have been fitted to individual breast cancer xenografts. This demonstrates that the growth rate of the untreated tumours is directly dependent only on the mitotic rate ($P < 0.001$), whereas tumour response to tamoxifen correlates most strongly ($P < 0.001$) with the relative balance between apoptosis and mitosis, as evidenced by the apoptotic:mitotic ratio.

Keywords: Apoptosis; Breast cancer; Gompertz; Tamoxifen; Xenograft

1. Introduction

Tumour growth or regression is regulated by the relative balance of cell proliferation and cell death. However, these processes may not be independent, with evidence that they are strongly correlated in both breast cancer [2] and other tumours [3,4]. It remains unclear, therefore, whether increased proliferation or a reduction in cell death primarily drives the pathogenesis of tumours. Furthermore, whilst the majority of anti-cancer therapies are thought to work by inducing apoptosis [5], tamoxifen has been reported to both increase cell death [6,7], and have anti-proliferative effects [8,9]. Thus, the relative importance of these two processes in the clinical efficacy of tamoxifen remains unclear. To address this issue, we have made use of the growth curves of individual xenografts, established in immunodeficient mice, and compared predicted tumour volumes with those observed during tamoxifen therapy.

The Gompertz function accurately models the growth of human tumour xenografts [10,11]. Indeed the application of such a growth function to the sequential volumes of an individual tumour has been suggested to permit a more detailed assessment of efficacy of drug therapy [12] but, to our knowledge, this approach has never been applied to tumours. We have previously shown that treatment with tamoxifen causes increased apoptosis and decreased proliferation in the ER-positive ZR-75 breast cancer cell line, established as xenografts in nude mice. Changes were evident within 7 days of therapy, before any significant differences were observed between the volumes of treated and control tumours [1]. In the current study, we have analysed individually the growth curves of these same xenografts, in order to ascertain whether it is the rate of cell proliferation or cell loss that best relates to tumour growth and response to tamoxifen therapy.

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0959-8049/01/$ - see front matter
PII: S0959-8049(01)00166-6

2. Materials and methods

2.1. Xenografts

Xenografts of the oestrogen-sensitive ZR-75-1 breast cancer cell line were established in both flanks of 58 female nude athymic mice [HsdOla:ICRF-nu, Harlan (UK), Bicester, Oxon, UK] bearing a subcutaneous (s.c.) slow-release oestrogen pellet (0.72 mg, released over 60 days, Innovative Research of America, OH, USA), as previously described [1]. Bidimensional tumour diameters were recorded, and tumour volumes were calculated as

$$\text{Volume} = \frac{\pi \times D \times d^2}{6},$$

where D was the larger of the two diameters.

Once tumour volumes reached approximately 0.25 cm^3, the animals were randomly assigned to either a treatment group in which a slow-release tamoxifen pellet (2.5 mg over 60 days, Innovative Research of America, OH, USA) was inserted adjacent to the oestrogen pellet; or a control group of seven mice in which no further pellet was inserted. The day on which this randomisation was performed was taken to be day 0.

Ten to twelve tamoxifen-treated tumours were excised on days 2 and 7, 14, 21 and 28; 11 untreated tumours were excised on day 0, and eight excised on day 28. All excised tumours were immediately snap frozen in liquid N_2, and then transferred to $-80^{\circ}C$ for storage.

2.2. Histology

After fixation in paraformaldehyde (Sigma Diagnostics, St. Louis, USA), and routine processing into paraffin blocks, 3 μm sections of each tumour were cut and mounted onto glass slides, and stained with Haematoxylin and Eosin (Sigma Diagnostics, St. Louis, USA). To assess the mitotic and apoptotic indices, 10 random high-power (×400) fields were examined by one observer using the HOME microscope [13], a computer-linked microscope that records which cells are marked, permitting later confirmation by a second observer. Any field containing or abutting an area of necrosis was not used. Apoptotic cells were identified by their morphometric appearance [14] and mitotic figures as defined by Baak [15]. The apoptotic and mitotic indices were defined as the ratio of the number of identified cells to the total number of tumour cells, and their ratio calculated, all as previously described [1]. In the few cases in which no mitotic figures were seen, including 3/12 tumours removed on day 7, the apoptotic:mitotic ratio was approximated as the total number of apoptotic figures identified.

Necrotic tissue was identified under light microscopy by the presence of cells that were morphologically necrotic, with or without adjacent haemorrhage. The total area of all regions of necrosis, and the total area of the section, were calculated using the HOME software.

2.3. Gompertz growth curves

The basic Gompertz function is

$$V(t) = V_0 e^{(\frac{\alpha}{\beta})(1-e^{-\beta t})}$$

where $V(t)$ is the tumour volume at time t, V_0 the volume at time $t = 0$, and α and β are parameters describing the initial growth rate (α/β) and the rate of decay from exponential growth (β). The theoretical maximum volume, when $t = \infty$, is equal to $V_0 e^{\alpha/\beta}$. It has been noted that this theoretical maximum volume appears to be roughly constant for a given species [16]. For the prediction of xenograft tumour volumes, one small modification is required, since the initial viable tumour volume (V_0) is unknown. Therefore a third variable (τ) is required, being the time difference from when the tumour volume was 1 mm^3 to when it was first measurable, thus eliminating the unknown V_0:

$$V(t) = e^{(\frac{\alpha}{\beta})(1-e^{-\beta(t+\tau)})}$$

Since this is not a linear function, the fit of the measured volumes V_I needs to be optimised. The most definitive comparison of different growth models as applied to tumour spheroids noted that not only was the Gompertz function as good as any other, but also that this was best done by using the method of least squares fitted to the *logarithms* of the tumour volumes rather than the volumes themselves [17]. This approach was therefore chosen, so that the equation becomes:

$$\ln(V) = \frac{\alpha}{\beta}\left(1 - e^{-\beta(t+\tau)}\right) \quad (1)$$

and the curve-fitting task is to minimise:

$$\sum_{i=1}^{n}\left(\ln V_i - \frac{\alpha}{\beta}\left(1 - e^{-\beta(t+\tau)}\right)\right)^2 \quad (2)$$

where V_i are the actual measured volumes.

In order to do this, advantage was taken of a general modelling package called ADAPT [18] which allows the actual function [i.e. Eq. (1)] to be user-specified. In all cases, only volumes recorded up to and including day 0 were used: for the untreated tumours, the succeeding volumes permitted assessment of the accuracy of the Gompertz curve to predict the subsequent xenograft growth.

The growth of an individual tumour will be due to the relative balance between the rate of proliferation and the rate of cell loss. There will be a necrotic proportion in each tumour: but since the contribution to the overall tumour volume of a necrotic rather than non-necrotic cell is not known, together with the fact that there are no data on the rate of clearance of necrotic cores of xenografts, this term was ignored. Indeed, it will be shown later, that the growth rate of the tumours did not correlate in any way with the size of their necrotic areas. Mathematically, the rate of change in its volume V will be increased by the proliferative proportion and decreased by the proportion undergoing apoptosis. These are estimated as the mitotic index (M) and the apoptotic index (A), respectively. Since we do not know the relative duration of mitosis and apoptosis, it is shown in the Appendix that if apoptotic cells are visible for ρ times as long as cells in mitosis, then the relationship between the growth rate of the tumour $\frac{dV}{dt}$, and the measured apoptotic and mitotic indices is:

$$\frac{dV}{dt} = V\left[M - \frac{A}{\rho}\right]$$

which re-arranges to $$\frac{1}{V}\frac{dV}{dt} = M - \frac{A}{\rho} \quad (3)$$

The term $\frac{1}{V}\frac{dV}{dt}$ corresponds to the relative growth rate, and can be calculated for each xenograft at any time point from the Gompertz curve fitted to the volumes up to and including day 0 for that tumour.

2.4. Response

Conventional clinical criteria of response are based on a comparison between the initial untreated volume and that subsequently observed whilst on treatment. However, this inevitably ignores any growth that might have occurred had the tumour been left untreated. Therefore, for the purposes of this study, response was defined as the percentage difference between the measured tumour volume, and the volume predicted from the growth curve had the tumour been left untreated:

$$\text{Response} = 100 \times \frac{\text{predicted volume} - \text{actual volume}}{\text{predicted volume}}$$

Thus, a response of 100% corresponds to a complete disappearance of the tumour, whereas a response of 0% equates to the actual and predicted tumour volumes being identical.

3. Results

3.1. Gompertz curve fitting for untreated tumours

Table 1 shows the individual growth curve parameters obtained by fitting a Gompertz growth curve to the

Table 1
Gompertz parameters for individual untreated ZR-75-1 breast cancer xenografts

	α	β	τ	R^2	Number of volumes available to day 0	% difference between actual and predicted day 7 volume
Day 0 tumours						
DAC001	0.4797	6.4E-02	11.8	1.00	3	N/A
DAC020	0.3677	7.7E-02	8.0	1.00	3	N/A
DAC030	0.3095	4.9E-02	20.6	1.00	3	N/A
DAC040	0.1523	9.1E-03	28.3	0.98	3	N/A
DAC041	0.4853	9.4E-02	2.3	0.99	3	N/A
DAC054	0.5072	7.0E-02	6.8	1.00	3	N/A
DAC055	0.3645	5.0E-02	15.4	1.00	3	N/A
DAC060	0.6248	8.9E-02	10.7	1.00	3	N/A
DAC066	0.4294	5.9E-02	5.0	1.00	6	N/A
DAC071	0.2229	2.1E-02	9.5	1.00	6	N/A
DAC094	0.2633	2.5E-02	8.5	0.99	6	N/A
Day 28 tumours						
DAC050	0.4772	5.7E-02	11.1	1.00	3	20%
DAC051	0.1191	5.5E-09	31.1	1.00	3	48%
DAC052	0.3701	3.9E-02	10.6	1.00	3	43%
DAC053	0.4456	6.7E-02	10.1	1.00	3	10%
DAC059	0.4846	6.1E-02	6.8	1.00	3	47%
DAC082	0.2546	3.5E-02	3.8	1.00	6	−10%
DAC087	0.1403	2.5E-09	5.3	0.97	6	12%
DAC096	0.0738	3.9E-09	14.2	0.86	5	−41%
Averaged	0.3264	4.2E-02	13.7	1.00	6	20%

N/A, not applicable.

Table 2
ZR-75-1 breast cancer xenografts removed after 0 days of tamoxifen treatment

	$\frac{1}{V}\frac{dV}{dt}$	Apoptotic index	Mitotic Index	Apoptotic:mitotic ratio
DAC001	0.055	0.0057	0.0014	4.1
DAC020	0.036	0.0063	0.0014	4.4
DAC030	0.038	0.0053	0.0002	27.0
DAC040	0.097	0.0063	0.0020	3.2
DAC041	0.049	0.0041	0.0005	9.0
DAC054	0.109	0.0096	0.0026	3.7
DAC055	0.079	0.0052	0.0021	2.4
DAC060	0.064	0.0077	0.0010	8.0
DAC066	0.051	0.0105	0.0016	6.6
DAC071	0.093	0.0135	0.0028	4.8
DAC094	0.097	0.0065	0.0029	3.8

volumes up to and including day 0. The R^2 values on day 0 and day 28, ranging between 0.97 and 1.00 (apart from a single tumour), indicate that in general an excellent fit was obtained. However, the more relevant test of 'goodness-of-fit' is the ability to predict the subsequent volumes for those tumours that were left to grow without tamoxifen treatment. Therefore, Table 1 also shows the percentage difference between the actual and predicted volumes on day 7 for the tumours allowed to grow beyond day 0 without tamoxifen therapy. Where only three tumour volumes had been recorded up to and including day 0, the Gompertz function tended to overestimate subsequent growth, whereas for the three tumours (numbers 82, 87 and 96) for which five or more volumes were available, subsequent volumes tended to be underestimated. Two individual curves are shown for example in Fig. 1: DAC053, with an excellent fit that persisted until day 28, and DAC096 with a poorer fit both up to and beyond day 7. Fig. 2 shows the average of the actual tumour volumes, the average of the individually fitted Gompertz curves, as well as the Gompertz fit to the averaged tumour volumes. This confirms that there is overall a reasonably

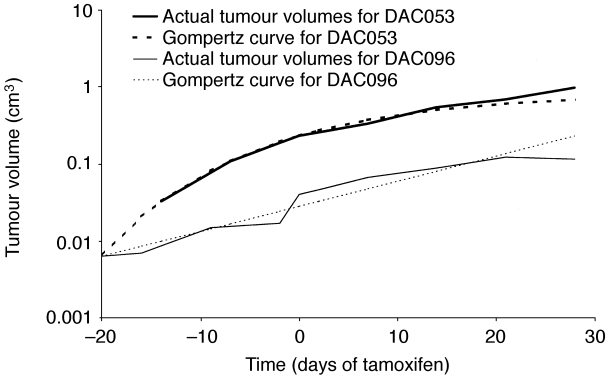

Fig. 1. Actual and predicted volumes for two untreated ZR-75-1 xenograpfts.

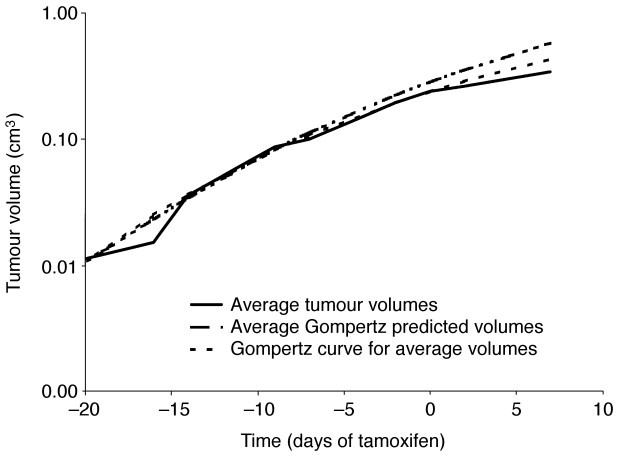

Fig. 2. Average actual and Gompertz growth curves for untreated ZR-75-1 xenografts.

good fit, but there is an impression that the Gompertz function might *overestimate* the subsequent volumes.

For the 11 tumours removed on day 0, individual apoptotic and mitotic indices, their ratio and the parameters for their unique Gompertz curve are given in Table 2, together with the relative tumour growth rate, $\frac{1}{V}\frac{dV}{dt}$. A significant positive correlation was found between the relative growth rate and the mitotic index, ($r = 0.835$, P value $= 0.001$) and is illustrated in Fig. 3. No such relationship was observed with the apoptotic index, the apoptotic:mitotic ratio, nor the proportion of the tumour that was necrotic. Furthermore, multivariate analysis with these four variables again found the only significant variable to be the mitotic index. This suggests that it is only the proliferative rate of the tumour that independently determines growth rate, and thus volume. Forcing the regression line through the origin gives the following equation:

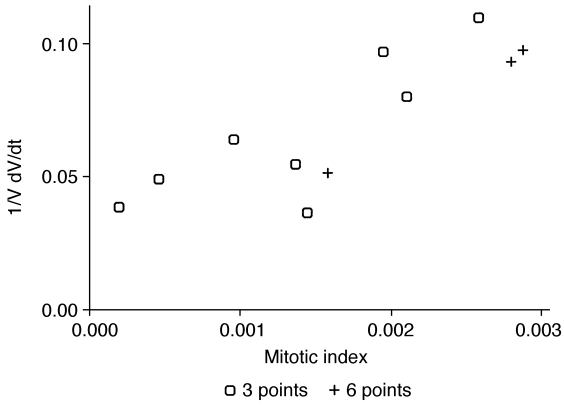

Fig. 3. Relative growth rate $\frac{1}{V}\frac{dV}{dt}$ for tumours removed on day 0, plotted against the mitotic index ($P = 0.001$), indicating the number of volumes available.

$$\frac{1}{V}\frac{dV}{dt} = 38.1x \text{ Mito}$$

There was a trend within these same 11 tumours for the apoptotic index to correlate with the mitotic index ($P < 0.08$), consistent with clinical studies [2] suggesting that the apoptotic rate is in part determined by the mitotic rate and thus why it may not independently determine the tumour growth rate.

3.2. Tamoxifen-treated tumours

The effect of tamoxifen on tumour growth is shown in Fig. 4. Although it is clear that the treatment induced tumour regression, the volumes of the treated tumours were not significantly smaller than the controls until day 14. Indeed, 12 (100%) and 6 (50%) of the tumours removed on days 2 and 7 were larger than they had been on day 0. Individual Gompertz growth curves were fitted to the volumes up to day 0 for all treated tumours.

For tumours removed on day 2, there were no significant correlations between the actual volume, predicted volume or response and the assessments of necrosis, apoptosis and mitosis (data not shown). However, for the tumours removed on day 7, there were significant correlations between tumour response and the apoptotic:mitotic ratio ($r = 0.829$, $P < 0.001$), the apoptotic index ($r = 0.803$, $P = 0.002$), and the mitotic index ($r = -0.766$, $P = 0.004$). There was no correlation with the extent of necrosis, nor between the actual tumour volume and any of these measures. The data pertaining to the strongest relationship, that with the apoptotic:mitotic ratio, are illustrated in Fig. 5, and the relationship appears to be non-linear, with an apparent plateau at higher values of the apoptotic:mitotic ratio. However, of the four points with the highest ratios, three had no mitotic figures in the counted fields, and hence the estimate (*vide super*) of the ratio may be an underestimate: the fourth tumour had 157 apoptotic figures, but only two mitotic figures so the confidence interval for the true ratio is quite wide.

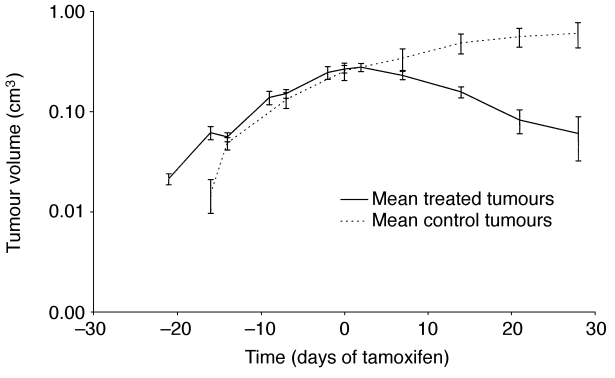

Fig. 4. Mean volumes of control and tamoxifen-treated ZR-75-1 xenografts.

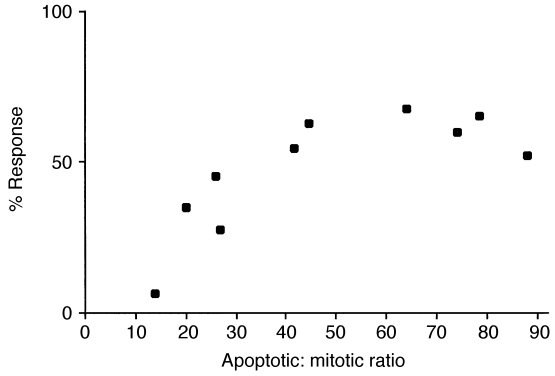

Fig. 5. Correlation between the apoptotic:mitotic ratio and the response after 7 days of tamoxifen treatment in ZR-75-1 xenografts.

3.3. Tamoxifen-treated tumours with stable growth

There were nine tamoxifen-treated tumours that had minimal change in tumour volume (defined as a change of $\pm 0.1\%$) over the 7 day period immediately prior to removal. The Gompertz parameters for these tumours, as well as the apoptotic:mitotic ratios, are shown in Table 3. For these tumours there was a significant correlation between the relative growth rate $\frac{1}{V}\frac{dV}{dt}$, and the apoptotic:mitotic index (see Fig. 6), with the equation of the regression line:

$$\frac{1}{V}\frac{dV}{dt} = 0.000513 - 0.000039 \text{ [apoptotic:mitotic ratio]}$$

$(P = 0.05)$.

From this, it can be shown (see the Appendix) that the relative duration of apoptosis to mitosis is 13.15 — which, if mitotic figures are assumed to be visible for 1 h, suggests that apoptotic cells are visible for around 13 h.

Confirmation of the relevance of this figure can be sought by applying it to Eq. (3) for each untreated tumour in turn. Knowing the observed apoptotic and mitotic indices for each tumour, the right-hand side can now be calculated and compared with the values for the

Table 3
Parameters for ZR-75-1 breast cancer xenografts with stable growth

	α	β	τ	$\frac{1}{V}\frac{dV}{dt}$	Apoptotic:mitotic ratio
DAC081	0.3720	4.8E-02	5.0	0.00000	14.0
DAC080	0.3096	7.1E-02	0.0	−0.00014	10.0
DAC025	0.1021	3.3E-07	35.8	0.00000	11.0
DAC043	0.3245	4.4E-07	13.1	0.00043	3.3
DAC093	0.0210	2.9E-09	119.2	−0.00057	13.0
DAC073	0.1212	1.7E-08	7.8	−0.00086	28.0
DAC063	0.0306	1.3E-08	47.4	0.00086	9.0
DAC061	0.3361	1.6E-01	0.0	0.00000	27.0
DAC028	0.3630	6.1E-02	5.5	−0.00060	27.0

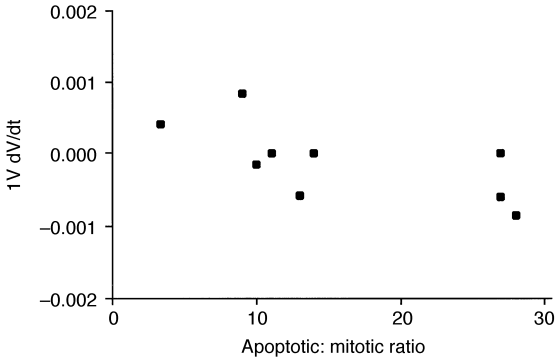

Fig. 6. Relative growth rate and apoptotic:mitotic ratio in tumours with minimal growth ($P=0.05$).

left-hand side ($\frac{1}{V}\frac{dV}{dt}$). For the 11 untreated tumours, there is a highly significant positive rank correlation ($r=0.846$, $P<0.001$). There was, however, no such correlation for the tumours removed after 2 or 7 days of treatment with tamoxifen.

4. Discussion

This study confirms that the growth of ZR-75-1 breast cancer xenografts is well described by the Gompertz function. Furthermore, it is the first study to report the fit of a Gompertz curve to individual xenografts without having made any assumptions about their average behaviour, in contrast for example to the work of the Copenhagen group who assumed that all tumours had the same maximal tumour volume [11]. For most tumours the fits are very good, as evidenced by the majority of the R^2 values being equal to 1. Since most continuous three-parameter functions can be fitted to three data points, it is more important to consider the fits in those tumours where four or more data points were available, as well as to consider the ability of the fitted curve to predict subsequent growth in those tumours not treated with tamoxifen. Assessing the predictive ability was only possible for the eight tumours in Table 1 that had been left to grow until day 28 without tamoxifen therapy. For the three tumours with more than three data points, two had very good fits; the third, tumour DAC096, had one of the poorest fits overall, but as it can be seen in Fig. 1, the Gompertz curve parallels the growth up to day 28. Indeed, the figure suggests that the problem may lie in the actual volume recorded on day −2. Predictive ability has been formally assessed only up to day 7, since it is at that time (before significant tumour regression was observed) that differences in apoptosis and proliferation were found [1]. This ability of the Gompertz function to model and predict xenograft tumour growth is consistent with the extensive prior literature [10,19–22].

The data confirm that for this particular model system, it is only the mitotic index that correlates with the relative growth rate, with no significant relationship noted for apoptosis or necrosis. It is of interest that the same conclusion was drawn by Baak's group in Amsterdam, studying the progression from pre-invasive to well differentiated carcinoma [23]. This is not to be interpreted as implying that these two mechanisms of cell loss have no effect on the tumour volume: clearly they must do. What the data suggest, however, is that the rates of apoptosis and necrosis do not independently contribute to the volume, and by inference may therefore be themselves determined by the rate of proliferation. However, in contrast to the sole importance of proliferation in determining the growth rates of these breast cancer xenografts, it can be seen from Fig. 5 that the strongest relationship between the change in growth seen after 7 days of tamoxifen is found with the apoptotic:mitotic ratio. Because of the design of this experiment, it was not possible to determine, in the tumours treated with tamoxifen for 7 days, the apoptotic and mitotic indices just prior to commencing tamoxifen. Thus the *change* in their ratio induced by tamoxifen could not be measured — but the observation that after 7 days of treatment it is the ratio that best correlates with response, rather than either index separately, suggests strongly that the rate of both processes are important in tamoxifen's efficacy within this model system. This conclusion is consistent with earlier observations from Jordan's group, in which tamoxifen treatment of xenografts was noted to cause a fall in labelling index (a measure of proliferation) [24] and a rise in cell-loss [25]. For clinical tumours, no comparative data are available, since it is not ethically acceptable to leave tumours untreated in order to determine the untreated growth rate. However, we have found that the change in the apoptotic:mitotic ratio after 3 months of tamoxifen correlates with the observed tumour response [26]. Further studies are therefore underway in women with breast cancer to test the hypothesis that a change in the proliferation:cell death ratio after a few days of tamoxifen predicts subsequent tumour regression, as suggested by the data in this study.

Those tamoxifen-treated tumours whose parameters are shown in Table 3 all developed a plateau in their growth, and therefore an estimate for the value of ρ in Eq. (3) can be obtained from their apoptotic:mitotic ratios. Fig. 6 confirms that there was a significant correlation between the apoptotic:mitotic ratio and the observed relative tumour growth rate. It is shown in the appendix that, by fitting a linear regression line, an estimate of 13.15 can be derived for ρ, suggesting that apoptotic figures are visible considerably longer than mitotic figures, which may have implications for studies that assess the effect of therapy on apoptosis. If the widely accepted figure of around 1 h is assumed for the

duration of mitosis, then these data suggest a value of 13 h for the duration of apoptosis, which accords with the value found using different methodology in a different model system [27]. However, it must be acknowledged that this value may not generalise for example to clinical tumours where there may be a higher chance of clearance by macrophages, etc. However, when this value was substituted back into Eq. (3), there was an extremely good correlation between the value of $\frac{1}{V}\frac{dV}{dt}$, as calculated using the actual apoptotic and mitotic indices for each untreated xenograft, and that derived from the Gompertz growth curve at the time of tumour removal. This is further corroborative evidence for the validity of the value of 13 h for the duration of apoptosis in this model system.

There are a number of possible criticisms of this model. Firstly, the model does not separately recognise any contribution to the tumour volume of non-breast cancer cells. However, it is unclear that their relative contribution to the volume is altered by therapy, and more sophisticated studies would be required to determine this. Furthermore, the assumption that they are not a significant determinant of tumour volume is consistent with other studies in clinical tumours [28]. Secondly, the rates of cell death and proliferation were assessed by morphology, and not by immunohistochemistry. However, the basic parameters in the model were the rates of acquisition and loss of cells, and there is no clear evidence that immunohistochemistry is more accurate in the measurements of these rates. For example, the proportion of cells that stain positive for markers like Ki-67 certainly reflects the proliferating fraction of the tumour. However, since these techniques identify cells at all stages of the cell cycle, this approach is also sensitive to any changes in the duration of G1 or S-phase that could occur with oestrogen-deprivation [29]. The use of the mitotic index, however, only relies on the duration of mitosis.

Xenograft studies provide a good model for human cancer because of the inherent reproducibility of a model system using genetically identical tumours. However, clinical tumours are characterised by phenotypic, as well as genotypic, diversity, and conventionally, reports of xenograft studies average or summate the data in order to minimise the effect of differences in individual tumour behaviour on the results. The advantage of individually modelling tumour growth is shown here by the fact that there was no correlation between the mitotic index and the actual tumour volume, in contrast to that reported for the individually-derived relative tumour growth rate. Similarly there was, for the tumours removed on day 7, no correlation between the apoptotic:mitotic ratio and the actual tumour volume or a conventional measure of response, whereas there was with a definition of response that incorporated the growth predicted to have occurred without any treatment. It should be noted that although individual tumour growth patterns have been recognised in this study, all the xenografts were established from the same original cell line. Nevertheless, this study has addressed the behaviour of individual tumours, and in so doing has confirmed that despite differences in growth rates, the same pattern of behaviour is seen in all tumours; namely that growth is driven primarily by the rate of proliferation, whereas response to tamoxifen relates to the relative balance between proliferation and apoptosis.

Acknowledgements

The authors were, at the time this research was conducted, all supported by the Imperial Cancer Research Fund, Lincoln's Inn Fields, London, UK. Two anonymous reviewers are thanked for bringing some relevant publications to our attention

Appendix

Since each cell that enters mitosis produces two daughter cells, then during a single time interval, the tumour will gain a number of new cells exactly equal to the number of cells that completed mitosis during that same time interval. Similarly, each time a cell completes apoptosis, then the tumour loses one cell, so that the cell loss rate equates to the number of cells completing apoptosis during that same time interval. Assuming that all tumour cells have the same volume, we have:

$$\frac{\Delta V}{\Delta t} = \text{Cell volume} \times (\text{number completing mitosis} - \text{number completing apoptosis})$$

where ΔV represents the change in total tumour volume, and Δt the time interval.

However, one cannot measure the total number of cells completing apoptosis and mitosis — only the proportion undergoing these processes in a sample of the tumour. Furthermore, the total number of cells multiplied by the average cell volume is by definition the total tumour volume V. Therefore the equation becomes:

$$\frac{\Delta V}{\Delta t} = V \times (\text{proportion completing mitosis} - \text{proportion completing apoptosis}).$$

This model does not allow for the contribution to the tumour volume of blood vessels, interstitial cells or necrotic tissue. However, since both the extent of the angiogenesis and necrosis may relate to the growth rate of the tumour, it may not be essential to model them independently.

In order to estimate the proportion of cells completing apoptosis and mitosis during a time interval, one has to extrapolate from the proportion undergoing these processes in the sample of tumour section examined. However, examination of a tumour at a single time point can not measure the total number of cells in the cell cycle — only those that are in any particular part.

Since this study has used morphological techniques, one can only identify the cells in mitosis. Thus, the measures obtained are the proportion of cells *visibly* in mitosis and apoptosis. When a tumour is at a stable volume, the number completing apoptosis and mitosis must be identical. This situation is shown in Fig. 7, and using a standard time interval equal to the duration of mitosis. In the example shown, the duration of apoptosis is taken to be twice that of mitosis, and it can be seen therefore that twice as many apoptoses are to be seen in any tumour section examined if the number completing each process in the time interval is the same, as must be the case when there is no net growth.

Hence, if ρ is the relative duration of apoptosis to mitosis (2 in the example shown in Fig. 7),

$$\frac{\Delta V}{\Delta t} = V \times [\text{proportion visibly mitotic} - (\text{proportion visibly apoptotic} \div \rho)],$$

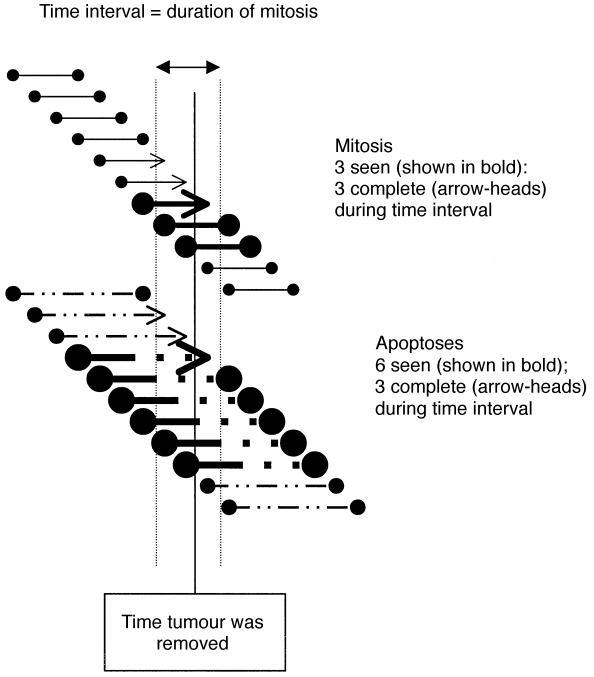

Fig. 7. The figure shows that the observed occurrence of apoptoses and mitoses depends on the rate at which these processes occur as well as their relative duration. In this example, the rates at which apoptosis and mitosis occur are the same, resulting in no net tumour growth. However, the duration of apoptosis is twice that of mitosis, so that the apoptotic index is twice that of mitosis when assessed by microscopy of a section removed at the time indicated.

but the proportion visibly mitotic is the mitotic index M, and the proportion visibly apoptotic is the apoptotic index A. In the limit as the time interval$\rightarrow$0, this becomes:

$$\frac{dV}{dt} = V\left[M - \frac{A}{\rho}\right]$$

When the tumour is in stable growth, $\frac{dV}{dt} = 0$. Thus we have, for a non-zero tumour volume,

$$\frac{dV}{dt} = V\left[M - \frac{A}{\rho}\right] = 0$$

$$\Rightarrow\ M = \frac{A}{\rho A}$$

$$\Rightarrow\ \rho = \frac{A}{M} = \text{apoptotic:mitotic ratio},$$

but we have from the experimental data for tumours with no net growth (see p. xxx):

$$\frac{1}{V}\frac{dV}{dt} = 0.000513 - 0.000039 \text{ [apoptotic:mitotic ratio]}$$

$$\Rightarrow\ 0 = 0.000513 - 0.000039 \text{ [apoptotic:mitotic ratio]}$$

$$\Rightarrow\ 0.000513 - 0.000039 \text{ [apoptotic:mitotic ratio]}$$

$$\Rightarrow\ \text{apoptotic:mitotic ratio} = 13.15 = \rho$$

Thus, using the data on the apoptotic and mitotic indices for tumours with no net growth, we derive an estimate of the duration of apoptosis being 13 times as long as that of mitosis.

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