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of tumour cell proliferation:
a comparison to experimental data

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Quantitative models describing the kinetics of tumour cell proliferation: a comparison to experimental data

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Pedigrees of cells from a mouse osteosarcoma line are analyzed. The generation time data of cells of 12 pedigrees is used to estimate the parameters in two transition probability models of the cell cycle.

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1. Introduction

Tumour growth is governed by rates of cell division and cell loss. In most solid tumours proliferating, quiescent, dying and migrating cells coexist, giving rise to a complex composition of cell populations. For a fundamental understanding of the kinetics of tumour growth a detailed knowledge of the proliferation of the individual cells is required.

The proliferative capacity of individual tumour cells can be investigated by several methods. One criterium for the analysis of cell proliferation is the incorporation of radioactively labeled nucleic precurses into the newly formed DNA of replicating cells. Quiescent or dying cells are not in the process of DNA synthesis and do not take up the radioactive label. By testing the DNA of individual cells using autoradiography, the fraction of activily dividing cells within a population can be determined at a specific time. The clonogenic assay, a second criterium for cell proliferation, provides information on cell growth at the end of a number of cell cycle periods. When an isolated cell, going through a number of cell divisions within a predetermined time interval, generates a clone of 50 cells, then that cell is judged to be actively proliferating. Dying or quiescent cells will not attain the required clone size.

This report describes the sequence of events that takes place in the time between the plating of a tumour cell and the observation of a clone at the end of a number of generation times. The data on cell proliferation have been taken from experiments published in Kooi *et al.* [4]. The evolution of cell clones has been recorded in detail through prolonged microcinematographic observation of isolated cells growing in culture. A large variation in growth patterns has been observed. In these experiments the kinetics of a perturbed cell culture was compared to the kinetic of a control culture. To analyse the data we applied two transition probability models of the cell cycle: the Smith-Martin model [6] and the model by Brooks-Bennett and Smith [2].

2. Experimental techniques and data handling

Cells from a mouse osteosarcoma line were incubated in vitro at optimal culture conditions, see [4]. Single cells attached to the bottom of the culture flask were selected for continuous microcinematographic observation. The growth of each cell was followed during a period of at least 7 average cell cycle times. Through the analysis, frame by frame, of the exposed film, a pedigree was constructed for each cell, representing the evolution of that cell into a clone. Cell divisions, changes in

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morphology, cell death etc. were coded and introduced at a position corresponding to the time of the event and to the family relation within the pedigree.

The pedigrees analyzed were obtained from two types of experiments, a control experiment in which cells were cultured without intervention from outside and an experiment in which a significant but nondamaging perturbation of the cell cycle was introduced through a change of culture medium during the second cell cycle.

The information contained in the pedigrees was transfered manually to the CDC CYBER 170-750 computer system at the Academic Computing Center Amsterdam. The data of each pedigree were subjected to several tests for internal consistency. This procedure proved to be effective in eliminating most typing errors. A coding system for cellular events has been developed that is easy to use and that is sufficiently flexible to provide for most of the observations recorded from the films exposed during these experiments. The coding system and the graphical construction of the pedigrees have been described in VAN DER HORST and GRASMAN [3].

3. CELL CYCLE TIMES

The control data were derived from 6 pedigrees of unperturbed cell populations. The generation time, Tc, of 218 cells from these pedigrees was recovered for analysis, see fig. 1. The Tc's of the remaining 7 cells were deleted because of anomalously long division delays. The average Tc was 783 min. with a standard deviation of 129 min. These values can be taken in an approximation of the generation times by a normal distribution. In the first 6 generations no dependence of Tc on the generation number was observed. In the 7th generation Tc rose sharply, but, because many of these cells did not divide during the film exposure, no average Tc could be determined.

In the perturbation experiments the cultures were reincubated with fresh medium after cell division. Again 6 pedigrees were analysed and the generation time data of the first five generations (117 cells) were used. None of the cells had an extremely long generation time nor was there any sharp rise in the generation time at the sixth or seventh generation. In fig. 2 it is seen that the average Tc varies with the generation number: the second generation cells have a larger Tc, which is positively correlated (r=.52) with the age at the cells of the moment of reincubation. The average cell generation time of the third generation is slightly larger than that of the fourth and fifth, which have about equal values. There is no correlation between the Tc's of third generation cells with the Tc's of the mother cells. Considering the 89 cells in the fourth and fifth generation we find an average cycle time of 591 min. with a standard deviation of 63 min.

4. TRANSITION PROBABILITY MODELS

From the review paper by BERTUZZI and GANDOLFI [1] we have chosen two transition probability models of the cell cycle and we have estimated the parameters from the data of the experiments described in the forgoing sections.

In the model of SMITH and MARTIN [6] it is assumed that the cycle can be split in an A-state with an exponentially distributed residence time and a B-phase with a normally distributed length, see fig. 3. In this three parameter model M_3 the probability density of Tc is the convolution of a normal distribution $\mathcal{N}(\mu,\sigma^2)$ with an exponential distribution Exp(λ). This convolution yields

$$f(Tc;\mu,\sigma,\lambda) = \lambda e^{-\lambda(Tc-\mu-\sigma^2\lambda/2)}\Phi\left(\frac{Tc-\mu-\sigma^2\lambda}{\sigma}\right)$$

with

$$\Phi(x) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{x} e^{-\frac{1}{2}\xi^2} d\xi.$$

The parameters μ, σ and λ follow from the equations

$$\frac{\partial S}{\partial \mu} = \frac{\partial S}{\partial \sigma} = \frac{\partial S}{\partial \lambda} = 0,$$

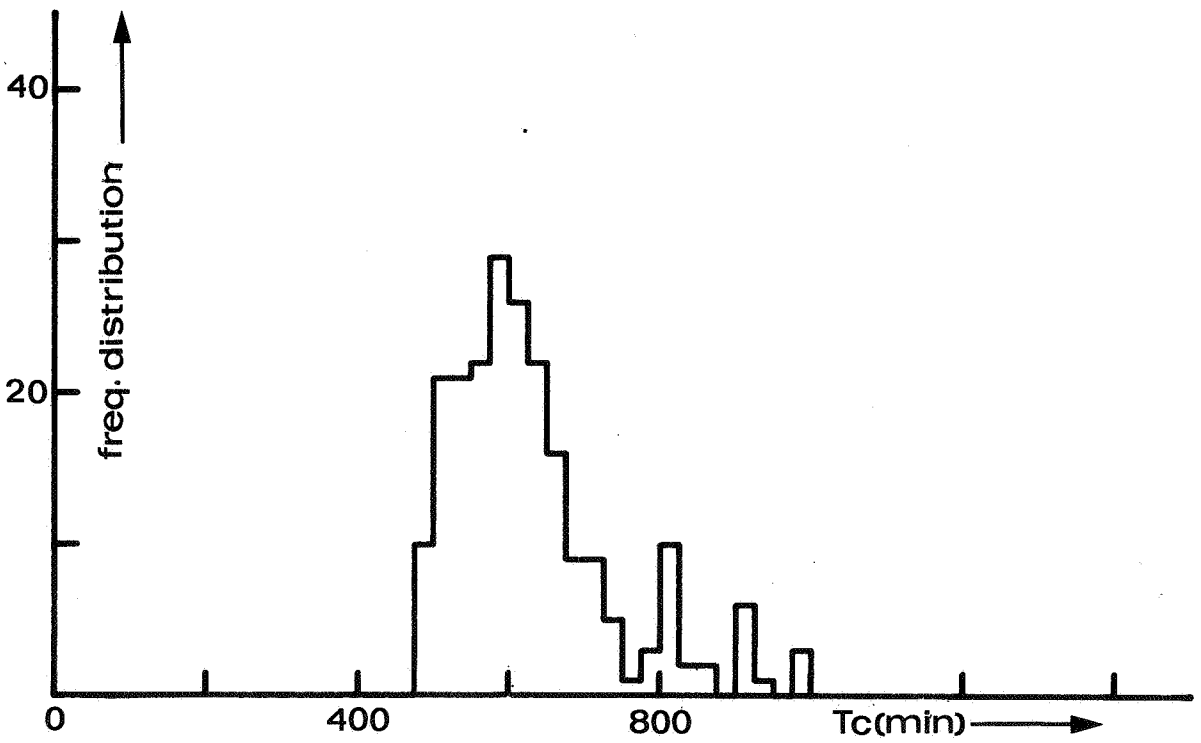

Fig. 1 Distribution of cell generations times Tc for 218 cells.

where S is the loglikelihood function

$$S = -\sum_{i=1}^{N} \log f\,(Tc_i;\mu,\sigma,\lambda)$$

and $Tc_i, i=1,...,N$ the generation times of the cells. In table I we give the results for the two experiments. The chi-square test for the difference between the model M_3 and the normal distribution $\mathcal{N}(783,129^2)$ of the preceding section yields for the unperturbed experiment ($N=218$):

$$\chi_1^2 = S(\mathcal{N}) - S(M_3) = 11.1$$

which has a p-value of 0.1%. Consequently, inclusion of an A-state with an exponentially distributed residence time means a significant improvement of the model.

From the registered Tc values we constructed a cumulative distribution (using 205 points). The theoretical cumulative distribution satisfies

$$F(Tc;\mu,\sigma,\lambda) = \int_{-\infty}^{Tc} f(t;\mu,\sigma,\lambda)dt.$$

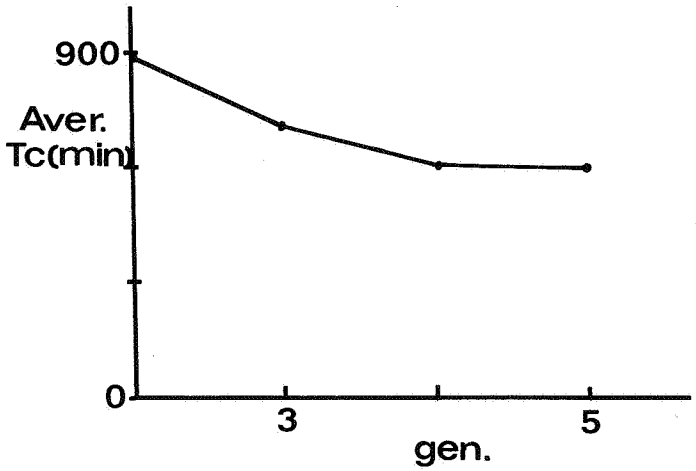

Fig. 2. Average cell generation time Tc as a function of the generation in perturbation experiment of reincubation with fresh medium in the second generation.

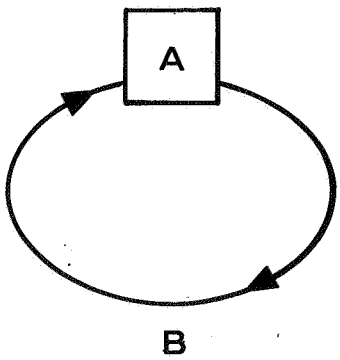

Fig. 3. The Smith-Martin model: the residence time in the A-state is exponentially distributed, the length of the B-phase is either fixed or normally distributed.

experiment	generations	N	μ	σ	λ
control	2-6	218	654	39	.0076
fresh medium	4-5	89	531	21	.0167

Table I. Estimated parameter values of the model M_3.

Then from this distribution we also estimated the parameters by the method of least squares. The same values for μ and λ were found; the standard deviation σ differed about 10% with the loglikelihood estimator of σ.

The second transition probability model, we considered, is from BROOKS, BENNETT and SMITH [2]. The α-curve satisfies

$$\alpha(Tc) = 1 \quad \text{for} \quad Tc < \mu,$$

$$\alpha(Tc) = \frac{1}{\lambda_A - \lambda_Q}\{\lambda_A e^{-\lambda_Q(Tc-\mu)} - \lambda_Q e^{-\lambda_A(Tc-\mu)}\} \text{ for } Tc > \mu,$$

where $\alpha(Tc) = 1-F(Tc)$. The following parameter values were found in the control experiment ($N=218$):

$$\mu=609,\ \lambda_A = .0083 \text{ and } \lambda_Q = .019.$$

For the sister-sister correlation coefficient we have

$$r = \frac{1/\lambda_Q^2}{1/\lambda_Q^2 + 1/\lambda_A^2}.$$

From the data in the control experiment we calculated $r=.55$. Consequently, λ_A and λ_Q should be about equal. In that case the α-curve is for $Tc > \mu$ determined by the quotient of two small numbers, which makes the curve quite sensitive to errors in the data. Thus this model is not suitable for the present study.

Since the residue of the M_3 model is just slightly smaller than that of the above model, we are led to conclude that the M_3 model yields a fit which can be expected from any reasonable model with 3 parameters. We therefore also reject the model M_3. Since the exponentially distributed residence time of the A-state meant a significant improvement of the model, we take the two parameter model M_2 of Smith and Martin with a B-phase of fixed length T_B as a starting point for our further investigations. Using the method of least squares to fit the cumulative distribution we obtain for the 218 cells of the control experiments the following values:

$$T_B = 645,\ \lambda = .0070.$$

Checking the goodness of fit for the two models M_2 and M_3 we consider 8 intervals for the cell generation time and determine the chi-square values:

$$\chi_4^2 = 15.0 \text{ and } \chi_5^2 = 17.3,$$

which indeed have about the same p-values.

5. Concluding remarks

In the cultures which we perturbed by a change of the culture medium, the Tc's were shorter than in the control cultures: taking only into account the fourth and fifth generation we find a reduction of the residence time in the A-state of about 50% and reduction of the length of the B-phase of about 15%.

In the control cultures we observed 7 cells with anomalously large division delays. These cells we deleted in the process of estimating the parameters in the transition probability models of section 4. In the data of pedigrees of irradiated cells (to be analyzed in a subsequent report), such cases occur more frequently. This is a strong indication that we have to introduce a quiscent state in our model of the cell cycle and that we have take into account a delay due to DNA repair. We refer to Rittgen & Tautu [5] for transition probability models with a quiscent state.

In our test of the model of Brooks, Bennett & Smith [2], the data of sister-sister correlations contained the necessary information to reject this model for cells of a mouse osteosarcoma line. In a next paper sister-sister correlation as well as cousin-cousin correlation will be analyzed in more detail, see Van Zoelen *et al.* [7].

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