The Use of Cell Proliferation Data in Modeling of Skin Carcinogenesis

by Annette Kopp-Schneider

A simple model for papilloma formation is used to analyze data from a mouse skin-painting experiment performed with NMRI mice. The results suggest that one of two conclusions may be drawn: Either the model fails to properly describe the growth behavior of papilloma cells or the model suggests that papilloma cells do not have growth advantage over normal cells, even during promotion.

A simple one-stage model for the formation of papillomas is applied to data from a mouse skin-painting experiment performed with NMRI mice. The data come from a standard initiation-promotion experiment where promotion was stopped at prespecified time points. In this experiment, four groups of 60 animals each were used with different lengths of promotion: 5, 10, 20 and 40 weeks. The groups were observed for 41 (5-week promotion experiment), 44 (10-week promotion experiment) and 52 weeks (20 and 40-week promotion experiments). The animals were treated with 100 nmole of the initiator 7,12-dimethylbenz(a)anthracene (DMBA), followed by twice weekly applications of 5 nmole of the promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Individual weekly papilloma counts were available, and papillomas were counted if their size exceeded 1 mm in diameter.

The model used here (Fig. 1) is a special case of a two-stage model for carcinogenesis with clonal expansion, which was first suggested by Kendall (1), Neyman (2), and Neyman and Scott (3) and is usually attributed to Moolgavkar and colleagues (4,5). The model considered here consists of two types of cells, normal cells and initiated cells. All cells are assumed to act independently. Growth kinetics for normal cells are not considered here because initiation is assumed to occur instantly, and normal cells are not of concern after the initiating event. Initiated cells are assumed to be subject to a linear birth and death process, with constant birth rates \( \beta_1 \) and death (differentiation) rates \( \delta_i \) during each experimental step, i.e., \( \beta_1 \) and \( \delta_i \) before promotion, \( \beta_2 \) and \( \delta_2 \) during promotion, and \( \beta_3 \) and \( \delta_3 \) after promotion stops. It is assumed that papillomas with diameter 1 mm contain more than \( M \) actively dividing initiated cells, and papillomas with a diameter smaller than 1 mm contain at most \( M \) actively dividing initiated cells. Information about this variable, \( M \), is currently unavailable and therefore the number has to be estimated from the papilloma count data.

Morris and Argyris (6) measured the cell-cycle time for basal layer cells of CD-1 mice treated with a single application of 200 nmole DMBA to be in a range of 121–175 hr (5–7 days). They also measured the cell-cycle time of basal layer cells treated with an additional single application of 17 nmole of TPA. The cell-cycle time depended on the time since application of TPA; 1 hr after treatment it was 16 hr, and 3 days after treatment it was 25 hr. For the purposes of modeling, a constant cell-cycle time of 6 days was assumed for un promotes initiated cells, and a constant cell cycle time of 20 hr was assumed for initiated cells under promotion. This can be translated into birth rates of \( \beta_1 = 6.94 \times 10^{-3} \) hr\(^{-1} \) = \( \beta_3 \) before and after the stop of promotion, and \( \beta_2 = 5 \times 10^{-2} \) hr\(^{-1} \) during promotion.

Figure 1. A one-stage model for papilloma formation, which is a special case of the two-stage model with clonal expansion.
The number of basal layer cells in the treated area of the skin can be estimated as $12 \times 10^6$ because the treated area of the skin has a surface of 12 cm$^2$ and a single cell of the basal layer occupies about 100 $\mu$m$^2$ (7). Therefore, it can be estimated that the process starts with $X = 12 \times 10^6$ normal cells. It can further be estimated that a papilloma of size 1 mm in diameter contains up to $5 \times 10^6$ cells. This number is not identical to the number of initiated cells present in a papilloma of size 1 mm in diameter because dead or differentiated cells may stay in the papilloma.

The remaining parameters were estimated from the data using maximum likelihood methods. The death rates were estimated in proportion to the birth rates, i.e., estimates were determined for $\alpha_1 = \delta / \beta_1$. The number of initiated cells produced by the initiating event, $\mu X$, and the number of initiated cells present in a papilloma of size 1 mm in diameter, $M$, was estimated.

Maximum likelihood methods were used to yield $\mu X = 83587$, $\alpha_2 = 1.0066$, $\alpha_3 = 1.0225$, and $M = 378$ under the assumption $\alpha_1 = \alpha_3$, assuming $\alpha_1 = 1$ yielded the same results with a minor change in the estimate of $\mu X$. Figure 2 shows observed and expected mean papilloma counts using the estimated model parameters. To illustrate the influence of small changes in $\alpha_2$, Figure 2 shows an additional set of curves obtained by restricting the range of $\alpha_2$ to be smaller than or equal to 1. This yielded estimates of $\mu X = 41305$, $\alpha_2 = 1$, $\alpha_3 = 1.0275$, and $M = 256$. From the plot, it can be seen that the fit of this model was insufficient, especially for the data from the experimental groups with long promotion.

For mathematical tractability, weekly papilloma

---

**Figure 2.** Plot of the observed and the expected papilloma counts from the 10-week and the 20-week promotion group. The best-fitting model had parameters $\mu X = 83587$, $\alpha_2 = 1.0066$, $\alpha_3 = 1.0225$, and $M = 378$; the alternative model had parameters $\mu X = 41305$, $\alpha_2 = 1$, $\alpha_3 = 1.0275$, and $M = 256$. (<--->) observed; (-----) best-fitting model; (-----) alternative model.
counts for each animal were assumed to be independent. Bootstrap methods were used to determine the distribution of the estimated parameters. For this purpose, 60 animals were sampled with replacement from each experimental group. For each animal, the whole stream of papilloma counts over time was taken. Parameters were estimated for this new collection of data, and the process was repeated 1000 times. The results are given in Table 1.

A sensitivity analysis was performed to investigate the influences of changes in the birth rates to the estimated parameters. In turned out that changes of the birth rates in their range (5–7 days for $\beta$, and 16–25 hr for $\beta_2$) had no significant impact on the parameter estimates.

It was further investigated how many experimental groups were necessary to yield satisfactory parameter estimates by evaluating the data from all single groups and all combinations of two or more experimental groups. It was apparent that two experimental groups were enough to yield qualitatively the same results as with the full set of data, and, surprisingly, the data from the 5-week and the 10-week promotional group already contained enough information to yield qualitatively the same parameter estimates as with the full set of data.

Standard initiation/promotion mouse skin-painting experiments had been evaluated earlier using a more complicated multistage model of carcinogenesis (8). These experiments suggested that only a few initiated cells are produced during initiation, and initiated cells have a growth advantage compared to normal cells. Translated into the terminology used here, $\alpha_2$ was estimated to be smaller than 1 in this earlier research. Using only the data from the initiation and promotion phase of the experiment, therefore, discarding the data from the stop-promotion phase yielded the same results as found in the earlier research. It was apparent that when using only this restricted data set, two sets of good parameters could be found, one similar to the early results and one similar to the results seen with the evaluation of the entire data set. The parameter set with $\alpha_2 < 1$ had a slight preference. Fixing the estimated parameters $\mu X$, $\alpha_2$, and $M$ and including the data from the stop-promotion phase to estimate $\alpha_3$ yielded a very unsatisfactory model fit for the model with $\alpha_3 < 1$. A more detailed analysis of the model parameters, a thorough discussion of the estimates, and a discussion of the reasons for the observed estimated parameters can be found in Kopp-Schneider and Portier (9).

On the basis of the model used here (Fig. 1), the data from the experiment suggest that at the initiating event, a large number of initiated cells are produced, papillomas contain only a small fraction of (actively dividing) initiated cells and the death/differentiation rate of initiated cells is higher than the birth rate, even in the presence of the promoter. This last conclusion means that the expected size of the initiated cell population is not increased by promotion. The results show the impact of modeling clonal expansion as a stochastic process; from the large number of initiated cells produced at initiation, only a few will survive and those will quickly grow to visible clones.

In consequence, there are two conclusions that can be drawn. Either it has to be concluded that the mechanism upon which the model is based fails to describe the growth behavior of initiated cells, or it has been concluded that initiated cells do not possess a growth advantage over normal cells. It should be stressed that these conclusions rely on the information yielded from the stop-promotion phase of the experiment. The results of this analysis suggest that more complicated experimental designs should be applied to gain further insight into the growth kinetics of initiated cells.

### REFERENCES

1. Kendall, D. G. Birth-and-death processes, and the theory of carcinogenesis. Biometrika 47: 13-21 (1960).
2. Neyman, J. A Two-Step Mutation Theory of Carcinogenesis. National Institutes of Health, Bethesda, MD, 1958.
3. Neyman, J., and Scott, E. Statistical aspects of the problem of carcinogenesis. In: Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics, University of California Press, Berkeley, CA, 1967, pp. 745-776.
4. Moolgavkar, S. H., and Venzon, D. J. Two-event models of carcinogenesis: incidence curves for childhood and adult tumors. Math. Biosci. 47: 55-77 (1979).
5. Moolgavkar, S. H., and Knudson, A. G. Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 6: 1087-1082 (1981).
6. Morris, R., and Argyris, T. S. Epidermal cell cycle and transit times during hyperplastic growth induced by abrasion or treatment with 12-O-tetradecanoylphorbol-13-acetate. Cancer Res. 43: 4935-4942 (1983).
7. Komitowski, D., Goerttler, K., and Loehke, H. Epidermal intercellular relationship during carcinogenesis and co-carcinogenesis as revealed by scanning electron microscopy. Virchow Arch. A2. E. Zeilplothol. 24: 317-333 (1977).
8. Kopp-Schneider, A., Portier, C. J., and Rippmann, F. The application of a multistage model that incorporates DNA damage and repair to the analysis of initiation/promotion experiments. Math. Biosci. 106: 139-166 (1991).
9. Kopp-Schneider, A., and Portier, C. Birth and death/differentiation rates of papillomas in mouse skin. Carcinogenesis, 13: 973-978 (1992).

### Table 1. Estimates of model parameters, their SDs and range obtained from 1000 bootstrap samples.

| Parameter | Mean | SD | Minimum | Median | Maximum |
|-----------|------|----|---------|--------|---------|
| $\mu X$  | 98.829 | 50.811 | 19.900 | 87.593 | 495.282 |
| $\alpha_2$ | 1.0067 | 0.0008 | 1.0042 | 1.0067 | 1.0094 |
| $\alpha_3$ | 1.0226 | 0.0012 | 1.0188 | 1.0225 | 1.0367 |
| $M$       | 381 | 19.62 | 319 | 380 | 450 |

$\mu X$ is the estimate for the number of initiated cells produced by the initiating agent at time of initiation, $\alpha_2$ is the estimate for the ratio of death rate over birth rate for initiated cells during promotion, $\alpha_3$ is the estimate for the ratio of death rate over birth rate for initiated cells after promotion stops, $M$ is the estimate for the number of actively dividing cells in a papilloma of diameter 1 mm.