1 Introduction

In the course of development of liver cancer, preneoplastic lesions occur which represent precursors of the malignant tumour. This is true for humans as well as for animals, regardless of the way in which the liver carcinomas develop, be it by induction through chemical agents, viral infections or radiation. The occurrence of focal hepatic preneoplasia has proven to be a valuable indicator of the ability of a treatment to cause liver cancer. Although the number of liver cancers induced in rats by chemicals is orders of magnitude lower than that of liver foci, there is a clear correlation between the two endpoints. Focal hepatic preneoplasia, therefore, represents a sensitive marker of carcinogenic response.

The finding that a compound alters the number of foci or their size distribution may give indications about the mechanism of action of the compound. A compound which increases the number of foci without changing their size distribution is called an initiator. A compound which increases the size of existing foci without changing their number is called a promoter. Usually compounds will have a combination of initiating and promoting activities. One question of interest to toxicologists is whether the initiating or the promoting potential of a compound is the predominant mode of action. Another question discussed among pathologists concerns the mechanism of liver focal lesion formation and their growth.

The evaluation of a quantitative study on preneoplastic liver lesions induced by an oncogenic agent involves the morphometric analysis of stained liver sections. The result of this analysis is one data set per liver section which contains the total area of the liver section and each individual focal transection area. Depending on the stain used for detection of focal lesions, only one or several phenotypes of foci can be distinguished. If more than one type of foci can be distinguished, the type of the foci is recorded in addition. As a rule, foci under a fixed minimum size are not recorded or discarded from the study.

Measurements are made in two-dimensional liver sections and inference about the reality in three-dimensional liver is limited by the stereological problem. This problem is described briefly by the fact that the probability of a focus to be cut increases with its size. Therefore, large foci will be overrepresented in liver sections compared to small ones, and the number of transections observed in a liver section depends on both the number of foci in the liver and their size distribution. Observations on number and size distribution of focal transections therefore cannot be directly related to three-dimensional reality. Due to the nature of the process of data collection and to the number of focal transections that can be observed, no stereological technique has so far been identified which can be applied to the two-dimensional liver sections to reconstruct the three-dimensional number and size distributions of focal lesions in the liver.

2 Stochastic models for hepatocarcinogenesis

Difficulties in the statistical analysis of hepatic preneoplasia can be eliminated by basing the analysis on a mechanistic model which describes the appearance and the change in volume of foci. Several models have been developed to describe foci data. All of these models assume
that foci appear randomly in space and time and use the concept of a Poisson process. The models are different as to how the change in volume and in phenotype of foci is described.

Two different hypotheses about the formation of preneoplastic lesions are discussed in the scientific community. The first hypothesis assumes that single cells are transformed to the next phenotype by a mutational event. This hypothesis is depicted in the upper path of Figure 1 for the case of three different types of preneoplastic lesions. The lower path shows the alternative hypothesis, called field effect hypothesis, that all cells in a preneoplastic lesion change their phenotype more or less simultaneously rather than by mutation of single cells.

![Figure 1. Two hypotheses about the formation and phenotypic changes of liver focal lesions. The upper path shows the mutation hypothesis and the lower path shows the field effect hypothesis](image)

The multistage model with clonal expansion

The most prominent biologically based models for carcinogenesis are the multistage models, which are formulated at the cellular level, describing the malignant transformation of normal cells as a process involving mutations, cell proliferation/differentiation and occasionally repair. They commonly assume that a cell has to go through \( k (>1) \) mutational changes and that intermediate cells are subject to a birth-death process. The basic assumptions for this type of model is that cells act independently.

As an exemplary multistage model we present here the fourstage model with clonal expansion. As depicted in Figure 2, a normal cell becomes malignant by sequentially passing through three intermediate (phenotypically different) cell types. Cells in the intermediate stages may proliferate according to a stochastic linear birth-death process with rate \( \beta_i \), denoting the intermediate cell type, they may die or differentiate with rate \( \delta_i \), or they may divide asymmetrically into one cell of the same type and one cell of the next type with rate \( \mu \beta_i \). The cells in the normal stage are assumed to stay constant in number and are not subject to the stochastic birth-death process. As in all current multistage models, the malignant stage is assumed to be absorbing. However, this stage will not be considered in the current application because data about carcinomas were not evaluated.

Multistage models represent interconnected birth-death processes and as such are treated with Markov process methods. Unfortunately the model presented here cannot directly be used for application to data because the distribution of number and size of cell colonies of different phenotypes cannot be derived analytically. Therefore an approximation of the model was derived that describes the transition process between the different types of cells by a Poisson process with intensity depending upon the mean number of cells in the previous
compartment. Details about the model and the approximation can be found in Geisler and Kopp-Schneider (2000).

The four-stage model describes the size of foci of altered hepatocytes in terms of number of cells. Under assumptions about the size of a cell and the packing of cells in a volume, the foci size distribution is translated into a distribution of foci radii in three-dimensional space.

The color-shift model

The field effect hypothesis is formalized in a model which describes the formation and fate of focal lesions in a three-dimensional situation. The centers of foci are generated according to a Poisson process. Foci start growing from the time point of formation onward according to a deterministic exponential law, initially starting from either a single cell or a whole cluster of cells. Growth occurs through clonal expansion of foci cells and/or recruitment of neighboring cells. It is assumed that the shape of every focus is spherical and therefore the size of a focus is given by its radius. When focal lesions are generated they all have the same phenotype. The phenotype changes sequentially over time, i.e. each focal lesion may pass through a sequence of phenotypes. The changes in phenotype are irreversible. The phenotype will be called 'color' with reference to the method of detection of focal lesions by staining tissue.

Details of the model can be found in Kopp-Schneider et al. (1998) and in Burkholder and Kopp-Schneider (2002).

3 Model-based evaluation of liver focal lesion data

Corresponding to the experimental data, the log-likelihood for both models consists of a sum over all individual animal contributions where each contribution involves the number of foci of every phenotype, the area of the liver slice and the size of all observed focal transections. The data obtained from experiments contains information from sections through a three-dimensional organ with three-dimensional preneoplastic lesions. For the application of both mechanistic models it is necessary to derive the two-dimensional size distribution of focal
transections from the three-dimensional size distribution prediction for foci of the models. As proposed by Moolgavkar et al. (1990), the Wicksell transformation is used to translate the size distribution of foci to a size distribution of focal transections.

A simplified version of the fourstage model, a one-stage model (cf. Moolgavkar et al. 1990) was applied to data from an experiment in which mice were treated with radiation (data not yet published). Here the question was whether radiation had any promoting activity. Although the number of observed foci was quite low, the model-based analysis gave clear indications that radiation had an effect on the growth or expansion of foci.

In another investigation, both the fourstage model and the color-shift model were applied to data from a carcinogenicity study in which rats were continuously treated with the carcinogenic compound N-nitrosomorpholine (NNM) (Weber and Bannasch 1994). The analysis was restricted to three types of foci. The Maximum-Likelihood parameter estimates for cell division rates in the fourstage model were not in the range of biologically plausible values. In addition, comparison of model fit which showed that the color-shift model explained these liver focal lesion data slightly better than the fourstage model, suggesting that for this type of preneoplastic foci the field effect hypothesis is a more likely explanation than the mutation hypothesis. This is intuitively appealing because the fourstage model predicts observation of many large early stage foci and few small later stage foci. Regarding the size distribution, the opposite is true for the color-shift model, which predicts many small early stage foci and few large later stage foci, this being the effect observed experimentally.

The example of the application of mechanistic models for hepatocarcinogenesis presented here shows their ability to contribute to cancer research. The models presented here can be treated with stochastic process theory and the quantities of interest for application to data can be derived analytically or calculated numerically. This allows to use formal Maximum-Likelihood methods for parameter estimation and for a comparison of model fit.

The models presented here are certainly oversimplifications of the biological process. A number of future developments are necessary, this concerns multistage models as well as the color-shift model. The growth process in the color-shift model needs to be described in a more realistic fashion and first investigations with altered growth process of the lesions have been performed.

References


