Supplementary Note

In this supplementary note we outline the details of the modelling approach to define the cell fate in clones induced to express *Dominant-negative Mastermind-like 1 (DNM)*.

1 Model of cell fate behaviour in the oesophageal epithelium

To model progenitor cell fate behaviour in the oesophageal epithelium we made use of a stochastic model introduced in Ref. *(31)*. Within this framework, progenitors make stochastic fate decisions in which the fate outcome of individual cell divisions is unpredictable, but defined by fixed probabilities. Although such models do not attempt to account for the potential influence of extrinsic factors in regulating both proliferative activity and fate choice, such factors have been shown to not impact significantly clonal evolution in the two-dimensional homeostatic system, pertinent to a stratified epithelium (*33*).

We suppose that oesophageal progenitors P, which are confined to the basal layer, divide with rate λ, giving rise to either two progenitors (*duplication*, $P + P$), one progenitor and one differentiated cell (*asymmetric cell division*, $P + D$), or two differentiated cells (*symmetric differentiation*, $D + D$). Differentiated basal cells D *stratify* with rate γ ($D \rightarrow S$) and suprabasal cells progressively S lose their nuclei with rate σ ($S \rightarrow \emptyset$). Cells without nucleus are not considered since individual cells cannot be resolved by the cytoplasmic clonal marker. All events are considered to occur stochastically. For simplicity, we consider Markov processes in which the timing between consecutive events (division/stratification) is statistically uncorrelated.

Applied to the normal oesophageal epithelium, previous studies have shown that the clonal fate data is consistent with balanced stochastic cell fate in which the frequency of cell duplication is perfectly balanced by symmetric differentiation *(7)*. To address clonal evolution of DNM mutant cells in the wild type (WT) background, we take the simplest generalisation of the model, allowing the frequency of symmetrical divisions to become unbalanced:

$$P \xrightarrow{\lambda} \begin{cases} P+P & \text{with Pr. } r(1+\delta) & \text{duplication} \\ P+D & \text{with Pr. } 1-2r & \text{asymmetric cell division} \\ D+D & \text{with Pr. } r(1-\delta) & \text{symmetric differentiation} \end{cases} \quad (1)$$

The parameter r regulates the balance between symmetric and asymmetric division, while δ defines the degree of bias towards proliferation. Any differentiated basal cell may then stratify:

$$D \xrightarrow{\gamma} S \qquad \text{(stratification)} \quad (2)$$

Finally, as cells move through the suprabasal cell layers, they progressively lose their nuclei:

$$S \xrightarrow{\sigma} \emptyset \qquad \text{(loss of nucleus)} \quad (3)$$

For $\delta = 0$, this model reduces to the established model of homeostasis *(7,31)* where loss of progenitors through differentiation is perfectly compensated by duplication. In a *non-homeostatic* tissue, however, cell fate may be biased, $\delta \neq 0$, leading to an overall gain/loss of progenitors. DNM-induced clones expand much more rapidly than WT clones (Fig. 1), indicating that cell fate is unbalanced. Nonetheless, the DNM clone size distribution retains a regular, unimodal structure and is very broad (Fig. 2b). We therefore propose that the principle mechanism of *stochastic* fate choice is retained for DNM clones, but with a potential bias $\delta \geq 0$.

To assess the validity of the model, and define the associated rates, we make use of the genetic lineage tracing approach defined in the main text. Following induction of control or DNM mice, a small fraction of cells express a hereditary fluorescent marker (YFP in control, GFP in DNM mice) and, for the latter, DNM. By staining fixed samples at subsequent time

points, we score the clonal progeny of individual cells by cell number, disaggregated into their basal (b) and suprabasal (s) cell content. From the statistical ensemble of clones, we determine the *clone size distribution (CSD)* which serves as the basis to define model parameters and challenge the model.

If we focus on the fate behaviour of the progenitor population alone, the model dynamics, Eq. 1 translates to a simple continuous time *branching process*, which has been extensively studied in the past (see, e.g., (*34*)) For the homeostatic system, the size distribution of surviving clones (i.e. clones that retain at least one progenitor) converges rapidly to the scaling form,

$$P(n) = \frac{1}{\bar{n}(t)} e^{-n/\bar{n}(t)}, \tag{4}$$

where $\bar{n}(t)$ represents the average number of progenitor cells per clone. Intriguingly, this form of the progenitor CSD is conserved even when the dynamics becomes unbalanced, $\delta > 0$ (*super-critical branching process*). However, the average progenitor cell numbers evolve differently:

$$\bar{n} = \begin{cases} 1 + \lambda r t & \text{balanced fate, } \delta = 0, \\ \exp(2\delta\lambda r t) & \text{unbalanced fate } \delta > 0 \ . \end{cases} \tag{5}$$

Thus, $\bar{n}$ diverges rapidly for unbalanced fate. Although such behaviour is qualitatively consistent with the observed expansion of DNM clones, it is clear that exponential growth is untenable and must become attenuated at some point, as suggested by the clone growth curve (Fig. 1c). We therefore expect that the model of unbalanced stochastic fate, based only on cell-intrinsic regulation, will only be applicable at early times following DNM mutation. Later, in section 4, we will discuss the transfer to long-time behaviour.

For the joint CSD of clones, including both progenitors and differentiated cells, analytical results are known in the balanced case (*35*), but the short-term dynamics for unbalanced fate cannot be usefully recovered in analytical form. Therefore, in the following, we use *stochastic simulations* to compute the CSD as predicted by the model dynamics. For this purpose, we make use of a Gillespie algorithm (*36*) to compute the stochastic evolution of model clones. The

inferred CSD can then be compared with that obtained by experiment. From this comparison, we derive the likelihood of the parameters using an algorithm defined in the following section.

2 Fitting procedure

2.1 Likelihood and Bayesian probability

When fitting the data by the model, we follow a maximum likelihood principle. The likelihood $L(\theta)$ of model parameters $\theta = (\lambda, r, \gamma, \sigma, \delta)$ represents the probability that the model with those parameters reproduces exactly the observed experimental data $\mathcal{D}$; $P(\mathcal{D}|\,\theta)$. Thus, according to the given data $\mathcal{D}$, the parameters θ^* with the maximum likelihood $L^* = L(\theta^*)$ are those which are most likely to reproduce the data. These parameters have therefore the highest predictive power for the observations and are chosen as the best fit. This approach is also justified by Bayesian statistics. The Bayesian theorem states that the probability – or better: certainty – of a model with parameters θ, given the data $\mathcal{D}$, is $P(\theta|\mathcal{D}) = L(\theta)P(\theta)/\int_\theta L(\theta)$, where $P(\theta)$ is the *a priori* certainty of the parameters without considering the data (*37*). We do not have any prior information. Thus, we assume that *a priori* all parameters are equally certain, $P(\theta) = const.$ This means that the most certain parameter value is exactly θ^*: $\max_\theta[P(\theta|\mathcal{D})] = P(\theta^*)$.

The model predicts the probabilities $p_{bs}(\theta)$ to observe clones with b basal cells and s suprabasal cells. The data is given in the form of the CSD $\mathcal{D} = \{f_{bs}\}_{b,s}$, where f_{bs} is the frequency of measured clone sizes with b basal and s suprabasal cells. Under the model assumptions, the probability to observe exactly f_{bs} times a clone with b basal cells and s suprabasal cells is $P(f_{bs}|\theta) \sim p_{bs}^{f_{bs}}$, not considering the normalisation. Since, in the model, the clones evolve independently from each other, the likelihood $L(\theta)$, i.e. the probability to observe the clone frequencies $\mathcal{D} = \{f_{bs}\}_{b,s}$ simultaneously, is $P(\{f_{bs}\}|\theta) \sim \prod_{b,s} p_{bs}^{f_{bs}}$. Accounting for the nor-

malisation, which assures that the probabilities sum to one, the likelihood is

$$L(\theta) = P(\mathcal{D}|\theta) = \frac{[\sum_{b,s} f_{bs}]!}{\prod_{b,s} f_{bs}!} \times \prod_{b,s} p_{bs}(\theta)^{f_{bs}} \quad , \tag{6}$$

which is a *multinomial distribution*. When fitting datasets from different time points $\mathcal{D}_1, \mathcal{D}_2, \ldots$ simultaneously, the likelihood for fitting the total data $\mathcal{D}$ is $P(\mathcal{D}|\theta) = \prod_t P(\mathcal{D}_t|\theta)$. With the results of simulation and Eq. 6, the likelihood of parameters θ can be computed and compared.

2.2 Likelihood distribution and maximum likelihood

To find the parameters with the maximum likelihood and their error margins, we determine the likelihoods for a wide range of parameters. For that purpose, we 'scan' the parameter space by simulating the model for a close-meshed ensemble of parameters θ and determine the likelihood $L(\theta)$ of each of them. The parameters $\theta = (\theta_1, \ldots \theta_5) = (\lambda, r, \gamma, \sigma, \delta)$ are varied independently from each other by a small difference $\Delta\theta_i$ ($i = 1, \ldots 5$) within the possible range of parameters $\theta_i^{\min}$ and $\theta_i^{\max}$. More precisely, the simulated parameters are the set $\Lambda = \{\theta_i^{\min} + j\Delta\theta_i \,|\, 0 < j\,\Delta\theta_i < \theta_i^{\max} - \theta_i^{\min}; i = 1, \ldots, 5\}$. For each run we simulated 150000 clones.

To find the maximum likelihood parameters we first performed a rough scan over a wide range of parameters $\theta_i^{\min(r)}, \theta_i^{\max(r)}$ but with large steps $\Delta\theta_i^{(r)}$ in order to get an estimate for the range of parameters where $L(\theta)$ is essentially non-zero. The corresponding values are displayed in Supplementary Table 1. Then the likelihood was computed taking finer steps $\Delta\theta_i^{(f)}$ within the restricted range of parameters $\theta_i^{\min(f)}$ and $\theta_i^{\max(f)}$, also displayed in Supplementary Table 1. The parameters θ^* with the maximum likelihood $L^* = P(\mathcal{D}|\theta^*)$ can then be extracted from the resulting likelihood distribution $L(\theta \in \Lambda)$.

2.3 Error margin of parameters

The maximum likelihood parameters are subject to different sources of noise in the data and simulations, mainly due to small numbers of clones, but also due to variations between animals.

parameter	$\theta_i^{\min(r)}$	$\theta_i^{\max(r)}$	$\Delta\theta_i{}^{(r)}$	$\theta_i^{\min(f)}$	$\theta_i^{\max(f)}$	$\Delta\theta_i{}^{(f)}$
cell division rate λ	0	$15\,w^{-1}$	$1\,w^{-1}$	$4.5\,w^{-1}$	$7.4\,w^{-1}$	$0.1\,w^{-1}$
symmetric division fraction r	0	0.15	0.01	0.03	0.1	0.005
stratification rate γ	0	$10\,w^{-1}$	$0.5\,w^{-1}$	$0.2\,w^{-1}$	$1.7\,w^{-1}$	$0.1\,w^{-1}$
loss rate σ	0	$10\,w^{-1}$	$0.5\,w^{-1}$	$0.2\,w^{-1}$	$1.6\,w^{-1}$	$0.2\,w^{-1}$
progenitor proliferation bias	0	1	0.1	0.6	1	0.05

Supplementary Table 1: Parameters for scanning the parameter space. For each combination of parameters in the set $\Lambda = \{\theta_i^{\min} + j\Delta\theta_i \,|\, 0 < j\,\Delta\theta_i < \theta_i^{\max} - \theta_i^{\min}; i = 1, \ldots 5\}$, the likelihood is determined by stochastic simulations and using Eq. 6.

We therefore determine the error margin of the parameters by classifying the parameters which are statistically acceptable[1]. For that purpose we define an *acceptable parameter set* Ω which contains all parameters whose likelihood is above a given threshold acceptance level, according to a *likelihood-ratio test* (*38*):

$$\Omega = \left\{ \theta \;\middle|\; \frac{L(\theta)}{L(\theta^*)} > \epsilon \right\} \quad . \tag{7}$$

Thus we accept all parameters whose likelihood relative to the maximum likelihood is larger than ϵ. It has been shown that this likelihood-ratio test is the most powerful test to classify sets of acceptable parameters (*38*).

The *acceptable intervals* for the individual parameters are then bounded by the maximum and minimum parameter values in this set, i.e. the lower acceptance limit of any parameter θ_i is $\theta_i^- = \min_\Omega\{\theta_i\}$, while the maximum acceptance limit is $\theta_i^+ = \max_\Omega\{\theta_i\}$. In the following these acceptable intervals will mark the error margins of our parameter estimation by the fitting and we will present the fit results for each parameter in the form $\theta_i = \theta_i^{*\,+|\theta_i^+ - \theta_i^*|}_{\,-|\theta_i^- - \theta_i^*|}$. In our estimations we choose an acceptance level of $\epsilon = 0.05$ which means that we accept any parameters that have at least 5% of the maximum likelihood.

[1]The term "acceptable parameter" is defined here in terms of *hypothesis testing*. If the hypothesis: "The model with the given parameters θ describes the data correctly" cannot be statistically rejected, the parameters θ are acceptable.

2.4 Confidence intervals of data points

Since the abundance of clones of a given clone size, f_{bs}, may be very small in the experimental data, it is subject to substantial statistical noise. In order to validate our model, we check if its predictions for each data point are plausible, within the *95%-confidence intervals*. This is the range which, in repeated experiments, would be covered by the 95% of outcomes closest to the average, for each data point. While we do not have the capacity to repeat the experiments an arbitrary amount of times, we can use our model to get an estimate for the confidence ranges. For that purpose we run the stochastic simulations a large number of N times repeatedly, taking the best fit parameters θ^* and the same total clone number as counted in the lineage tracing experiments. Then, for each basal clone size b (or joint basal/suprabasal sizes (b,s)) we select the subset of $0.95 \times N$ outcomes which are the closest to the average (we choose $N = 1000$). The range from the lowest value of this subset to the largest one gives the confidence interval.

3 Fitting analysis: short-time clonal dynamics

With this background, we turn now to the analysis of the DNM clonal data focusing on early times post-induction. Making use of the fitting algorithm defined above, from a fit to the *joint* CSD at 7 and 10 days post-induction, we obtain the parameters with maximum likelihood (*best-fit parameters*) shown in Supplementary Table 2 together with their acceptable intervals. In Figures. 2e,f and Supplementary Figures. 2a,b, the joint CSDs from experiment and simulation with best-fit parameters are compared at 7 and 10 days post-induction. In Figures. 2b and Supplementary Figure 2c the corresponding basal CSDs, giving the frequencies of basal cells per clone, are also shown and compared.

Although the fits of the model to the data show generally good agreement, there are some small but significant discrepancies. In particular, with the control data (Fig. 2e and Supplemen-

parameter	best fit value	control *(7)*
cell division rate λ	$6.0^{+0.4}_{-0.7}$ per week	1.9 ± 0.1 per week
symmetric division fraction r	$0.055^{+0.02}_{-0.01}$	0.1 ± 0.01
stratification rate γ	$0.8^{+0.3}_{-0.2}$ per week	3.5 ± 0.5 per week
loss rate σ	$0.6^{+0.7}_{-0.4}$ per week	1.0 per week
progenitor proliferation bias δ	$1.0^{+0}_{-0.1}$	0

Supplementary Table 2: Best fit parameters for the two data sets, 7 and 10 days post-induction.

tary Fig. 2a), clones with a single basal cell show a small departure from the model prediction. Such a deviation is likely to reflect the Markovian nature of the model dynamics, which allows for a very broad distribution of cell cycle times. If the cell division time has a natural upper limit, the model will overestimate the frequency of small clones. Although such "synchrony" correlations will influence the short-term dynamics, such effects will become erased from the clonal record after multiple rounds of division. However, with the slow cycle rate of the progenitors in WT (ca. once per 3.5 days on average), such effects remain visible in the experimental data at 10 days post-induction. Significantly, with the more rapid cell cycle rate of DNM progenitors (ca. once per day on average), these effects are invisible.

Further small deviations are visible in the shape of the suprabasal distribution of DNM clones. Once again, this deviation, which is visible only at the shorter time 7 day time point, is likely to reflect the Markovian approximation that fails to account for potential "maturation effects", which may delay stratification once a basal cell has exited cycle.

Finally, the Markovian approximation, which allows cells to divide without any refractory period following division, leads to the appearance of unfeasibly long tails of the clone size distribution. To avoid the potential to "over-fit" these tails, we restrict the range of data to a maximum clone size ($b_{\max} = 26, s_{\max} = 12$) in the joint CSDs. This choice includes all data in the joint CSD (10 days post-induction) except sparse regions in the tail with data points that

are separated from the bulk by more than one point (b, s) with $f_{bs} = 0$. To check whether the cut-off in the fitting range had any significant impact on the results, we also fitted the full data set, without the cut-off. The resulting basal CSD is shown in Supplementary Figure 2e. It can be seen that this also gives a reasonable fit with parameters that do not depart much from the primary fit. But one may note that the fit with the cut-off is superior in matching the bulk of the data.

3.1 Comparison of DNM and control clones

In comparing the results of the analysis for the DNM clones and the control, several striking differences emerge:

Unbalanced cell fate: From the fit, we find that $\delta = 1$ is the most likely parameter, which means that the symmetric differentiation channel appears to be completely suppressed, leading to the functional "immortalisation" of progenitors. Although this finding alone does not completely rule out $P \to DD$ divisions, the complete absence of pure suprabasal ("floating") clones in the two considered data sets provides further support for the complete suppression of this channel. Later, in section 3.3.2, we quantify this argument to show that the $P \to DD$ divisions, if they occur at all, must be very rare at the considered early time points.

Then, with $r = 0.055$, we find that 11% of divisions result in duplication and 89% in asymmetric divisions, which means that the total fraction of PP divisions has not changed significantly by DNM expression. Furthermore, Notch-inhibition by DNM does not suppress differentiation through asymmetric cell division. Instead the fit suggests that putative symmetric differentiation events result in asymmetric division.

So, in summary, the most likely cell fate decision rules in DNM clones are

$$P \to \begin{cases} P + P & \approx 10\% & \text{duplication} \\ P + D & \approx 90\% & \text{asymmetric cell division} \\ \cancel{D + D} & & \text{symmetric differentiation} , \end{cases} \tag{8}$$

while in the WT the corresponding ratios are $P \to PP$ at 10%, $P \to PD$ at 80% and $P \to DD$ at 10% *(7)*.

Suppressed stratification: The stratification rate γ is much smaller (0.8 per week) than for WT (3.5 per week).

Accelerated cell division: Finally, the cell division rate is significantly enhanced in DNM clones (6 per week vs. 1.9 per week for WT).

3.2 Model predictions

To gain further confidence in the validity of the model, we can make use of the fit from the 7 and 10 day clonal data to predict the CSD of the 15 day time point. In doing so, we find that the model is able to reproduce the DNM clonal data (Supplementary Fig. 2d), with predictions that lie within the confidence interval.

For later times, no clonal data is available, since from 15 days on clones become so large that they start to merge. We can nonetheless compare the model predictions for the average basal cell number with the clonal area measured in the experiments. With a measured induction frequency of 1 per 530 ($\pm$ 120) cells, when the average number of basal cells per clone is $\bar{n}$, the area fraction covered by all DNM clones is $\bar{n}/530$. From a comparison of the model prediction with experiment (Supplementary Fig. 2f), we obtain excellent agreement up to 15 days post-induction, after which deviations between model and experiment start to develop. This departure, which reflects a sub-exponential growth of clones, indicates a change in fate behaviour at longer times.

Notably, the deviation between model and experiment occurs at around the time when clones start to merge. On the other hand, WT cells at the edge of DNM clones stratify faster than in the control (Fig. 3), which indicates that WT cells do not resist expansion of DNM clones. These observations suggest that, in the first 15 days following induction, clonal expansion is

unrestricted and proceeds with constant rates.

3.3 Consistency checks

3.3.1 Total clone size distribution

With a model dependent on 5 parameters $\delta, \lambda, r, \gamma, \sigma$, it was necessary to use the full range of data to fit the model. However, in finding that the stratification and cell loss rates of the DNM clones are small, it is evident that cell loss does not play a significant role at early times post-induction, $t \ll 1/\sigma$. Moreover, the results above show that $P \rightarrow DD$ divisions are effectively erased altogether at short times. Therefore, if we focus on the *total* CSD, the short-term dynamics will be fully specified by just two parameters, λ and r. In this case, applying the same statistical procedure, we obtain the best-fit parameters $\lambda = 5.4$ per week, and $r = 0.065$. With these parameters, the comparison of the model and data (Supplementary Fig. 2g) shows good agreement within the confidence range. The consistency of these results with the previous findings provides further evidence in support of the model, Eq. 8.

3.3.2 Inhibition of symmetric differentiation

When a clone loses all of its progenitor cells by symmetric differentiation and the differentiated cells stratify, the clone becomes detached from the basal layer. These "floating clones", which are seen in WT, provide direct evidence of symmetric differentiation following division. Such events are strikingly absent in the analysis of DNM clones at 7 and 10 days post-induction, even when further efforts were made to search for such events (following the study of an additional 320 clones at 10 days). Although these findings do not allow symmetric differentiation to be rigorously ruled out in DNM, we can give an upper bound for its probability.

In Supplementary Table 3 we computed by simulation the expected frequency, β, of floating clones for different values of δ, taking the best-fit values for other parameters. A floating clone, defined by $b = 0$ basal cells and $s > 1$ suprabasal cells, emerges with probability $\beta = \sum_{s>1} p_{0s}$.

δ	β(7 days)	β(10 days)	Prob(no floating clone)
0.95	0.085%	0.19%	0.26
0.9	0.19%	0.36%	0.072
0.89	0.21%	0.41%	0.053
0.88	0.23%	0.45%	0.039

Supplementary Table 3: Probability for absence of floating clones for different values of δ (simulating 10^6 clones, with other parameters corresponding to those listed in Supplementary Table 2). For $\delta = 0.88$ this probability is below 0.05, thus within a 95%-confidence level any value of $\delta \leq 0.88$ can be excluded.

The probability of having no floating clones at all, as observed, is

$$\mathrm{Prob}(\text{no floating clone}) = (1-\beta_{7\mathrm{d}})^{n_{7\mathrm{d}}}(1-\beta_{10\mathrm{d}})^{n_{10\mathrm{d}}} \ , \tag{9}$$

where $\beta_{7d,10d}$ is the probability for floating clones 7 days and 10 days after induction, respectively. Here $n_{7d} = 300$ and $n_{10d} = 570$ represent the total clone numbers recorded at respective times. In Supplementary Table 3 the expected frequencies and probabilities to find no floating clones are displayed. For $\delta = 1$ this probability is one, while it declines for lower δ, dropping below 0.05 around $\delta = 0.88$. Choosing an acceptance level of 5% as before, a probability of less that 0.05 means that the corresponding parameter is out of acceptance interval. Therefore we conclude that the minimal value for the proliferation bias is $\delta_{\min} \approx 0.88$, corresponding to a maximal fraction of symmetric differentiation events to be $0.7\,\%$ on a 5%-acceptance level.

3.3.3 EdU as a clonal marker

Administration of *ethinyldeoxyuridine (EdU)*, which is taken up during S phase and is retained after cell division, provides a second lineage tracing marker to follow sub-clones within GFP-marked clones. This system can therefore be used to validate the predicted clonal dynamics.

To implement this program, we administered EdU to animals 7 days after induction with DNM and recorded the average number of EdU+ cells per DNM clone after 24h, 48h and 72h (see Materials and Methods). In contrast to genetic labelling, we can not be sure that all EdU+

time	EdU cells/clone (model)	EdU cells/clone (data)
48 h	3.03	3.28 ± 0.16
72 h	4.16	3.96 ± 0.28

Supplementary Table 4: Average cell number of EdU+ cells per clone at different times after administration showing model predictions and lineage tracing data. The error margins represent the statistical standard deviation of the data. The measured cell number after 24 hours has been taken as the initial condition.

cells in a clone originate from a single EdU+ cell. Therefore, to address the model prediction of clonal dynamics, we took the measured cell numbers at 24h EdU incorporation as an initial condition, and analysed the prediction of the model at 48h and 72h. On the basis of the observed division and loss rates, we supposed that no EdU cells are lost over the three days of observation.

According to the model dynamics, since only progenitor cells can divide, the average EdU+ cell number per clone n evolves in time t as

$$\frac{dn(t)}{dt} = \lambda\, n_p(t) \tag{10}$$

where $n_p(t) = n_p(0)\exp(2\delta\lambda r t)$ denotes the average progenitor cell number (cf. Eq. 5). At 24h post-administration on average $n(t_0 = 24h) = 2.33 \pm 0.11$ cells were measured. While the number of progenitor cells at 24h cannot be measured directly, we can infer $n_p(t_0 = 24h)$ by making use of the model: assuming the first cell division to occur 8 hours after EdU incorporation (the approximate average time between S-phase and completed cell division *(28)*[2]), we have $n_p(t_0) = (1 + (1 + \delta)r)\exp(2\delta\lambda r(t_0 - 8h))$, where $(1 + (1 + \delta)r)$ is the average number of progenitor cells after the first cell division. With this expression, Eq. 10 can be integrated. The comparison of the model with the measured data is shown in Supplementary Table 4. With the deviations between experiment and theory always within two statistical standard deviations (95% confidence interval), the best-fit parameters accurately predict the results

[2]Note that the time between EdU incorporation and cell division may vary. However, the final result is not very sensitive on the exact numerical value of this offset.

of the EdU tracing experiment.

4 Long-term clonal evolution

In section 3.2, we presented evidence that the model of unbalanced cell fate, valid at short-times post-labelling, must become adjusted at late times. By one year post-induction, virtually all epithelial cells express DNM without developing lesions. This suggests that the tissue attained a new steady state where cell fate has returned to balance. However, does this balance involve progenitor loss and replacement, or do all cell divisions lead to asymmetric fate outcome?

To monitor fate dynamics at one year after induction, we can again make use of the EdU assay as a clonal marker (see Materials and Methods). EdU was incorporated and connected EdU+ clusters were recorded 48 hours later. Due to high labelling frequency, not all clusters can be assumed monoclonal. However, a certain subclass of EdU+ clusters can be unambiguously classified as monoclonal and can be used for clonal analysis. In particular, since EdU is incorporated during S-phase, we know that each connected EdU+ cluster must have derived from a progenitor (or progenitors) and, after 48 hours, each of these progenitors must have divided at least once. Therefore, EdU+ clusters must contain at least two cells and any cluster that originates from two or more initial progenitor cells must have at least four cells. Thus, clusters consisting of only two or three cells can be considered as unambiguously monoclonal.

Using the same basic paradigm for progenitor cell fate, we can then infer the model parameters by solving the Master equation describing the time evolution of the probability of each cluster composition. Once again, with just a 48 hour chase, cell loss can again be neglected. If we define p_{AB} as the probability to find a two-cell cluster (doublet) with one $A = P, D, S$ cell

and one $B = P, D, S$ cell, we have

$$\begin{aligned} \frac{dp_{DD}}{dt} &= -2\,\gamma\,p_{DD} & \frac{dp_{PD}}{dt} &= -(\gamma+\lambda)\,p_{PD} \\ \frac{dp_{PP}}{dt} &= -2\,\lambda\,p_{PP} & \frac{dp_{DS}}{dt} &= 2\,\gamma\,p_{DD} - \gamma\,p_{DS} \\ \frac{dp_{PS}}{dt} &= \gamma\,p_{PD} - \lambda\,p_{PS} & \frac{dp_{SS}}{dt} &= \gamma\,p_{DS} \end{aligned} \tag{11}$$

The probability of three-cell clusters does not need to be treated explicitly, since it follows from normalisation of the probability distribution. This set of differential equations can be solved analytically. As initial condition we choose the state after the first cell division at time $t = t_0$, when the doublet compositions reflect the cell fate decisions. Hence at $t = t_0$ we have $p_{DD} = r$, $p_{PP} = r$, $p_{PD} = 1 - 2r$ and all other probabilities are zero. Although the time t_0 may vary between cells, this does not affect the prediction of r. Integrating these equation, we obtain

$$\begin{aligned} p_{DD}(t) &= e^{-2\gamma(t-t_0)}\,r & p_{PD} &= e^{-(\gamma+\lambda)(t-t_0)}(1-2r) \\ p_{PP}(t) &= e^{-2\lambda(t-t_0)}\,r & p_{DS} &= 2\,e^{-\gamma(t-t_0)}\,(1-e^{-\gamma(t-t_0)})\,r \\ p_{PS}(t) &= e^{-\lambda(t-t_0)}(1-e^{-\gamma(t-t_0)})(1-2r) & p_{SS} &= (1-e^{-\gamma(t-t_0)})^2\,r \end{aligned} \tag{12}$$

In the EdU assay, we recorded the number of EdU+ doublets and three-cell clusters and counted the relative frequencies f_{BB}, f_{BS} and f_{SS} of having each combination of basal (B) and suprabasal cells (S). Equating the relative frequencies with the corresponding model probabilities gives $f_{BB} = p_{DD} + p_{PD} + p_{PP},\ f_{BS} = p_{DS} + p_{PS},\ f_{SS} = p_{SS}$, a set of equations that can be solved uniquely for r, λ and γ (when substituting the solutions, Eqs. 12).

For the control animals we counted 39 PP-, 41 PD-, and 6 DD-doublets out of 136 clusters, giving the frequencies $f_{BB} = 0.29$, $f_{BS} = 0.30$ and $f_{SS} = 0.04$. Estimating $t_0 = 8$ hours as before (section 3.3.3), we obtain the solution for the control, [3]

$$r = 0.155 \pm 0.06, \quad \lambda = 2.1 \pm 0.4 \text{ per week}, \quad \gamma = 3.2 \pm 0.4, \text{ per week} \tag{13}$$

[3] Error margins result from solving the equations for $f_{AB} \pm$ one standard deviation of the counts, and applying linear error propagation.

(Note that r is in fact independent of t_0.) These values are, within the confidence intervals, consistent with the literature values *(7)*, providing a benchmark for the validity of the scheme.

For the DNM-induced animals, we counted 20 PP-, 50 PD-, and 8 DD-doublets out of 199 clusters, giving the frequencies $f_{BB} = 0.10$, $f_{BS} = 0.25$ and $f_{SS} = 0.04$. With $t_0 = 8h$ we get

$$r = 0.08 \pm 0.03, \quad \lambda = 4.3 \pm 0.6 \text{ per week}, \quad \gamma = 5.4 \pm 0.4, \text{ per week} \tag{14}$$

Again the solution for r is independent of t_0. This result shows that symmetric differentiation is reinstated, with a ratio comparable to the control within error bars. The cell division rate is lower than at early times but still significantly higher than in the control. Furthermore, the stratification rate, which was significantly depressed at short times, is also increased and in fact becomes higher than in the control. These results are consistent with the observation that thickness of the epithelium in DNM animals is comparable to WT after one year, considering that a higher cell division rate must be compensated by enhanced stratification to retain normal thickness.

References and Notes

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