The modulatory effect of cell–cell contact on the tumourigenic potential of pre-malignant epithelial cells: a computational exploration

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Malignant development cannot be attributed alone to genetic changes in a single cell, but occurs as a result of the complex interplay between the failure of cellular regulation mechanisms and the presence of a permissive microenvironment. Although E-cadherin is classified as a ‘metastasis suppressor’ owing to its role in intercellular adhesion, the observation that it may be downregulated at a premalignant stage is indicative of additional roles in neoplastic development. We have used an agent-based computational model to explore the emergent behaviour resulting from the interaction of single and subpopulations of E-cadherin-compromised cells with unaffected normal epithelial cells within a monolayer environment. We have extended this to investigate the importance of local tissue perturbations in the form of scratch-wounding, or ablation of randomly-dispersed normal cells, on the growth of a single cell exhibiting E-cadherin loss. Our results suggest that the microenvironment with respect to localized cell density and normal/E-cadherin-compromised neighbours is crucial in determining whether an abnormal individual cell proliferates or remains dormant within the monolayer. These predictions raise important questions relating to the propensity for individual mutations to give rise to disease, and future experimental exploration of these will enhance our understanding of a complex, multifactorial pathological process.

1. Introduction

The development of cancer in a healthy individual is a complex process, involving multiple processes and factors, which can rarely be traced to a single initiating event. Indeed, cancer may be regarded as the result of a complex interplay between genetic mutation and microenvironment [1,2].

Boutwell [3] postulated two distinct stages in skin carcinoma promotion: \textit{initiation}—the conversion of normal cells to a potentially malignant phenotype, and \textit{promotion}—propagation of the latter by proliferation (of dormant transformed cells) to form visible tumours [3]. This two-stage process was exemplified in studies where carcinogen was applied directly to the skin of nude mice. Following subsequent wounding of the skin containing the initiated cells, tumours were observed to form along the line of the wound, revealing tissue injury to be an important factor in promoting the propagation stage [4]. Similarly, in tongues of hamsters exposed to carcinogen, squamous cell carcinomas (SCCs) developed only in animals where the tissue at the site of application was subsequently excised [5]. The relevance of these findings to human carcinogenesis was demonstrated by Dayal and colleagues, who reported an increased incidence of oral epithelial dysplasia and SCC in oral submucous fibrosis (OSF) patients where there was also traumatic injury (mostly caused by sharp teeth) to the oral mucosa, compared with a second OSF group where there was no evidence of injury [6].
Tissue damage has also been shown to act as a tumour-promoting factor at a more systemic level. Dolberg et al. [7] developed an experimental model in chickens, in which virally transformed cells were injected and secondary tumours developed at a distal wound site created by the removal of a pre-positioned clip. It was shown that the growth of these secondary tumours was dependent on the injury caused by removal of the clip rather than on the presence of a foreign body. These observations are relevant to man—for example, marjolin ulcers are a particular form of aggressive SCC that occurs at sites of previously traumatized skin, such as chronic open wound ulcers [8].

The role of tissue injury as a trigger to local tumourigenesis may be related to the associated release of growth promoting signalling factors, but also the abrogation of normal intercellular contact. As early as the 1960s, the difference in the ability of normal and transformed cells to impede growth of neighbouring cells via contact inhibition was recognized [9]. Later, key experimental research involving the transplantation of mouse embryonic cells to extraterritorial sites in adults identified intercellular contact as important in determining the fate of these cells [10]. In 1990, it was demonstrated that normal human keratinocytes (NHKs) grown in physiological calcium concentrations would inhibit the growth of a transformed keratinocyte cell line in co-culture [11]. Other disruptions of normal epithelial architecture may provide a permissive environment for tumourigenesis, such as pregnancy-related reorganization of mammary gland [12] or loss of cell polarity [13].

Research effort has focused on identifying mechanisms involved in suppressing transformed cell growth. In epithelial tissues, intercellular contacts are mediated by E-cadherin, a cell-surface-expressed transmembrane protein that, in physiological calcium concentrations, binds to E-cadherin expressed on an opposing cell membrane. These homotypic interactions are critical in forming initial adherens contacts between cells in developing tissues or cell cultures, thereby allowing more established intercellular structures, such as tight junctions and desmosomes, to develop. A number of studies have implicated E-cadherin as playing a critical role in maintaining intra-epithelial latency in transformed epithelial subpopulations in culture. For instance, work by Alt-Holland and Zhang demonstrated that the ability of NHKs to inhibit the growth of transformed epithelium derived cells when seeded at a ratio of 12:1 was dependent on the presence of E-cadherin-mediated contacts. This inhibition could be abrogated by UV irradiation, which induced apoptosis preferentially in the normal cells [14]. E-cadherin has also been implicated in the spread and metastasis of transformed cells: for example, the abrogation of E-cadherin expression by transformed NHK cells in three-dimensional organotypic culture resulted in invasion of these cells into the underlying stroma [15]. The same group went on to demonstrate that loss of E-cadherin from the same cell type was associated with progression from low- to high-grade carcinoma [16]. Our own work has revealed that E-cadherin has a complex interplay on growth regulatory pathways—for example, by promoting proliferation in low-density cultures of normal human uro-epithelial cells, which is mediated through the PI3K-Akt pathway [17].

Computational models provide a convenient framework within which it is possible to build a virtual representation of a biological system, and can be used to test hypotheses that can later be explored experimentally. Traditionally, models have been based on mathematical equations that provide an abstract representation of averaged cell behaviour. However, when the focus is on the exploration of heterogeneous systems, and, in particular, the role of interactions between individual cells within the system, the ability to represent single entities is required. This feature is implicit to the paradigm known as agent-based modelling, which has been applied to study an increasing number of cell-based systems. Individual agents representing biological cells are placed in a specified virtual environment and allowed to interact according to pre-programmed rules (e.g. including cell cycle progression, quiescence and division, migration and simple physical interactions). Each agent executes these rules according to its internal state and interaction with its immediate environment, which may include both extracellular factors and adjacent cells. There is no concept of overarching control in such models, meaning that model simulations produce emergent behaviour that arises solely from the behaviour and interactions that evolve at the cellular level.

Agent-based modelling has previously been applied to study various aspects of tumour growth and cancer biology, including tumour-induced angiogenesis [18], invasive brain tumours [19] and competing processes of cell proliferation and death in solid tumours [20]. More recent publications have integrated multiscale approaches, often extending cellular-level models to include variations in the genetic or phenotypic profile of individual cells [21] or the action of chemotactants, nutrients and the role of microvasculature [22]. In at least one case, model parameters have been chosen to represent patient-specific tumour growth [23]. Although many of the examples above have included the concept of intercellular adhesion, the role of this factor in determining the interplay of subsets of cells has not been explicitly explored in any detail.

By contrast, Ramis-Conde et al. [24,25] used a multiscale model of E-cadherin/β-catenin signalling to demonstrate that the loss of cell–cell adhesion in a small area of a confluent cell population could give rise to a ‘wave’ of cell detachment propagating from the initial site of disruption. The mechanism postulated was that intracellular release of sequestered β-catenin through loss of intercellular contacts acts as a transcription factor to upregulate expression of genes responsible for promoting migratory behaviour, ultimately resulting in an epithelial–mesenchymal transition. The potential modulation of cell proliferation as a result of loss of intercellular adhesion was not explicitly explored in this case.

In a previous publication, we used agent-based modelling to explore how a subpopulation of epithelial cells exhibiting loss of E-cadherin and seeded with normal cells at low density can impact the overall growth characteristics of the entire population, and, more specifically, may influence the growth rate and migratory behaviour of the non-transformed subset [26]. In this paper, our aim was to use the same individual-based modelling paradigm to explore the role of cell–cell contact in defining the phenotype (behaviour) of cells with tumourigenic potential. In addition, unlike previous agent-based explorations of aspects of cancer biology, which primarily relate to tumour growth or invasion in a confluent tissue or monolayer, we were specifically interested in simulating how local tissue injury influenced the fate of E-cadherin-compromised cells.
within the population. We consider a ‘single-hit’ scenario where loss of E-cadherin expression affects the ability of a single cell to form stable adhesion contacts, but with all other characteristics remaining unchanged.

Initially, we explore the effect of spontaneously transforming different numbers of cells in a pre-existing monolayer, and the effect of the configuration of these cells (i.e. distinct colonies compared with randomly dispersed cells). We then explore the potentially more physiologically relevant situation of a single transformed cell in a confluent monolayer that is then exposed to a micro-environmental disturbance in the form of scratch wounding, or the spontaneous removal of a subset of normal epithelial cells, representing cell death induced by a chemical or toxic insult.

Our results indicate that disruption of cellular contacts alone is sufficient to induce the clonal expansion of E-cadherin-deficient cells in most experimental cases. We also show that the expansion rate and ‘maximum clonal potential’ (the number of progeny arising from a transformed cell during a given time interval) are determined by the microenvironment of the transformed cell in terms of the cell density of surrounding normal cells and, where relevant, the proximity to an injury site.

2. Methodology

As stated earlier, we have used the paradigm of agent-based modelling—a methodology that has the advantage of representing individuals in a population, allowing the explicit inclusion of heterogeneity [27]. Each individual is spatially resolved and represented by an equivalent virtual entity that follows a set of pre-programmed rules. In our simulation, each virtual entity represents an individual biological cell, which is programmed to display either a normal or significantly reduced amount of cell surface E-cadherin (labelled as EC-N and EC-A cells, respectively), and the rules relate to the biological behaviours of cell cycle progression, cell division, intercellular bonding and cell migration.

We have described the construction of each rule set in detail and justified the parameters used in our generic model elsewhere [26]. All rules and parameters used here are identical to those described previously and we include only an overview of the model here.

Briefly, agents in the model represent individual urothelial (bladder epithelial) cells. This choice of cell type reflects our background research interest, but parameters could be modified to represent any other epithelial cell type. The agents are initially distributed randomly across a 1 mm² substrate in a growth medium environment that contains a fixed, uniform concentration of calcium ions. The latter enables us to represent any other epithelial cell type. The endogenous E-cadherin parameter is converted to a functional parameter according to the concentration of exogenous calcium (which is fixed at model initialization). This endogenous E-cadherin expression of a cell may ultimately determine its own growth arrest, or influence that of its immediate neighbours. Briefly, the endogenous E-cadherin parameter assigned to each individual cell is converted to a functional E-cadherin parameter according to the concentration of exogenous calcium (which is fixed at model initialization). This accounts for the fact that exogenous calcium is required in order to allow E-cadherin molecules on adjacent cell membranes to interact, thus giving rise to stable intercellular adhesion [28,29]. When any two cells come within 5 μm of one another (representing an estimate of lamellapodia length), a probability of stable contact formation is calculated, based on the functional

The model is incremented in fixed time steps, according to the order shown in figure 1. Cells may migrate, form transient or stable (E-cadherin-mediated) contacts and progress through the cell cycle according to an internal clock. Following the memory update of every agent according to the rules described below, a numerical algorithm is used to eliminate or minimize cell overlap that has arisen owing to cell growth, division and migration. This sub-model returns new positional information to the agent model. It should be noted that the purpose of this algorithm is to eliminate overlap only, and the concepts of active cell migration and adhesion are dealt with entirely within the agent model. Further details of this algorithm and all other rules and parameters can be found in Walker et al. [26].

Our virtual experiments examine the influence of compromised intercellular adhesion via reduced E-cadherin expression on the growth of affected EC-A and unaffected EC-N cells within the monolayer. Hence, the rules that define intercellular adhesion and cell cycle control are important in determining the outcome of the simulation. Figure 2 shows a flow chart illustrating the order of processes within the model by which the endogenous E-cadherin expression of a cell may ultimately determine its own growth arrest, or influence that of its immediate neighbours. Briefly, the endogenous E-cadherin parameter assigned to each individual cell is converted to a functional E-cadherin parameter according to the concentration of exogenous calcium (which is fixed at model initialization). This accounts for the fact that exogenous calcium is required in order to allow E-cadherin molecules on adjacent cell membranes to interact, thus giving rise to stable intercellular adhesion [28,29]. When any two cells come within 5 μm of one another (representing an estimate of lamellapodia length), a probability of stable contact formation is calculated, based on the functional

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**Figure 1.** Schematic showing simulation process.
E-cadherin parameter values of the two cells. A random number is then generated, and a stable bond formed if the calculated probability is greater than this number, thus making it more likely that stable cell contacts form in physiological than in low exogenous calcium concentrations.

The cell cycle rule set contains a checkpoint for cell cycle arrest that is linked to multiple intercellular contacts. In mid–late G1, a cell undergoes a check and is permitted to progress to S phase and ultimately mitosis only if it has had any cell contact (stable or otherwise) for a minimum period of 4 h, but currently has less than four, stable intercellular contacts. The former requirement was chosen to represent the concept of cell cycle modulation via juxtacrine signalling mechanisms, explored in a recent study [30], while the latter represents contact inhibition of growth.

Cells that fail to pass the checkpoint enter G0 or quiescent phase where they remain indefinitely, until a sufficient number of the contacts are broken to allow cell cycle re-entry. An additional rule forces G0 entry in the case where high intercellular density has forced a cell to ‘round up’ to the point where it has acquired a minimum permitted radius in the x–y plane. This rule assures eventual growth arrest even when stable intercellular contacts are compromised (e.g. in the case of a low exogenous calcium environment), as is observed in experiment [28]. Again, full details of the rules and relevant parameters are given elsewhere [26].

Figure 2. Process by which endogenous E-cadherin influences intercellular bond formation and cell cycle progression.

2.1. Model initialization

The purpose of this investigation was to examine the role of the cellular microenvironment and specifically cell–cell contact on the tumourigenic potential of a single, or subset of, E-cadherin null cells that arises within a confluent monolayer of cells with normal E-cadherin expression. This required execution of a pre-simulation, whereby an initial population of 200 normal (EC-N) cells were randomly distributed across a two-dimensional substrate and allowed to expand until visual confluence was reached. All cells were initialized in G0, but rapidly re-entered the cell cycle and proliferated to fill the space available. To allow for the fact that there was a continued increase in cell number after visual confluence was observed (as is seen experimentally in cell culture), all subsequent simulations were started from the model configurations saved at 125 iterations (approx. 85% maximum confluence), 150 iterations (approx. 95% maximum confluence) and 175 iterations (approx. 100% maximum confluence). In order to separate valid predictions from artefacts associated with stochasticity in the model, six randomly generated replicate starting conditions were used, and each simulation was executed for 125, 150 and 175 iterations in order to generate a set of random confluent starting conditions for the heterogeneous EC-N/EC-A simulations.

For each of the virtual experiments outlined below, either a single, or subset of dispersed or clustered cells, was selected and switched to the EC-A phenotype by changing the memory parameter representing surface expression of E-cadherin protein from a value of 1 to 0.01. The simulation was then run for up to a further 200 iterations, or until all cells entered the quiescent phase of the cell cycle (G0) owing to contact inhibition or overcrowding, resulting in no cell divisions for a period of at least 25 iterations. On the basis of previous simulation of the agent model to explore growth and scratch wound repair of normal epithelial cell populations in monolayer culture [31,32], the introduction of mutated EC-A cells into the model was used to examine a number of potential scenarios. For each virtual experiment, an appropriate control simulation was carried out, as described in each of the following subsections.

2.1.1. Experiment 1: exploring the effect of initial EC-A cell dispersal

In this case, models were initialized at confluence as described earlier, then a subset of cells switched as follows: increasing numbers of cells representing 1, 10 or 25 per cent of the existing cell population were either selected at random, or in the form of a circular colony located at the centre of the monolayer. The amount of surface-expressed E-cadherin of these selected cells was then switched from the previous normal level (= 1) to a significantly reduced level (= 0.01). All other parameters remained unchanged and the simulation was restarted. The number of cells and the cell cycle status (i.e. phase and potential quiescence) of all normal and compromised cells was recorded at each iteration.

2.1.2. Experiment 2: exploring the effect of scratch wounding

In this case, a scratch wound of either 250, 500 or 750 μm was created diagonally across the centre of each confluent model, and a single cell selected for ‘mutation’ at d minimum cell radii (d = 10 μm) from the wound edge in the upper left quadrant of the monolayer. The surface-expressed E-cadherin of the latter was then instantaneously switched from normal (= 1) to significantly reduced (= 0.01). In the case of the 250 and 500 μm scratch wounds, d, = 3, 15 and 30, and for the 750 μm wound, d = 3 and 15 (larger values of d would have risked incurring edge effects). The location for the mutation event was chosen so that the cell was located approximately in the centre of the quadrant (along an axis perpendicular to the wound edge), also in order to minimize any potential edge effects. The simulation was then restarted and the wound observed to close, as previously described in Walker et al. [32]. The number and cell-cycle status (i.e. phase and/or quiescence) of all normal and compromised cells were recorded at each iteration.

Control simulations were run for each value of and starting cell density. In each control simulation, the phenotype of a cell in the same position as for each experimental simulation was switched, and the simulation restarted without a wound being created.

2.2.3. Experiment 3: exploring the effect of removal of a subset of normal cells

Model initialization to generate a confluent monolayer of normal cells was carried out as described already. A single
cell close to the centre of the monolayer was then randomly selected, and its endogenous E-cadherin level switched from normal (¼ 1) to severely reduced (¼ 0.01). A random selection of normal cells representing the following fractional values (k) of the pre-existing normal cell population [k = 0.01, 0.05, 0.1, 0.25, 0.4 or 0.6] was then instantaneously removed from the model, representing cell loss from the tissue (e.g. due to death or apoptosis). The removed cells were selected randomly, and were not clustered in a particular region. The simulation was then restarted and cell numbers and individual states of all normal and compromised cells were recorded at each iteration.

Controls from the wound simulation above with d = 3 (so switched cells were distant from the model edges with no wound being created) were used as controls for these simulations.

3. Results

3.1. Experiment 1: exploring the effect of initial EC-A cell dispersal

As shown in figure 3, spontaneously switching 1 per cent of the population from a normal to an E-cadherin deficient phenotype had little effect on the growth of normal cells, and there was limited expansion of the EC-A population. However, when the switched cells constituted 10 per cent of the population, then there was an effect on growth, but both the nature and the magnitude of the effect were dependent on the spatial configuration of the switched cells. When the transformed cells were dispersed, the latter population

Figure 3. Growth curves obtained from virtual experiment 1. (a) Total EC-N cells when subpopulation of cells mutated at iteration 125. (b) Total EC-N cells when subpopulation of cells mutated at iteration 150. (c) Total EC-N cells when subpopulation of cells mutated at iteration 175. (d) Total EC-A cells when subpopulation of cells mutated at iteration 125. (e) Total EC-A cells when subpopulation of cells mutated at iteration 150. (f) Total EC-A cells when subpopulation of cells mutated at iteration 175. (Online version in colour.)
underwent a moderate expansion, and the growth rate of the remaining EC-N cells was enhanced compared with the control. However, when the transformed cells were present as a colony, the growth of the EC-A cells was further enhanced to the relative disadvantage of the EC-N population. When the transformed fraction was increased to 25 per cent, these effects were increased. No differences in the migratory characteristics of the two populations were observed (data not shown).

Figure 4 shows a series of screenshots produced at different time points during the simulation when cells were switched to the EC-A phenotype in a single colony constituting 1, 10 and 25 per cent of the total confluent population, and also for the case where 1 per cent of the population was switched randomly. Expansion of the 10 and 25 per cent transformed colonies is apparent, whereas the limited expansion for the 1 per cent case is not clearly visible.

3.2. Experiment 2: exploring the effect of scratch wounding

Figure 5 shows the growth curves for EC-A cells when an initial mutation of a single cell takes place at increasing distances from a 250, 500 and 750 μm scratch wound created after simulating the growth of a normal population for 150 iterations. It can be seen that in the case of the 250 μm scratch wound (representing removal of approximately 25% of the normal cell population), in all test cases where the original EC-A cell is positioned at $d = 3$ cell radii ($\approx$30 μm) from the wound edge, there was EC-A clonal growth, which was found to be statistically significant relative to control simulations ($p < 0.001$). In all cases, the mutated cell underwent at least four rounds of division post-wounding. At larger values of $d$, clonal growth appeared more variable, with five of six simulations showing at least three rounds of division in the case of $d = 15$, and four of six for $d = 30$.

However, in these cases, growth was not statistically significant relative to controls.

In the case of the 500 μm wound, all mutations resulted in statistically significant clonal growth irrespective of the initial position relative to the wound edge. In the majority of simulations, cells typically underwent five to six rounds of division, resulting in final numbers of EC-A cells, which were approximately twice those obtained in the 250 μm case, reflecting the additional space available for expansion associated with the larger denuded area. Analysis
The response of a subset of EC-N cells was also studied in detail, for comparison with the mutated cell response. For each simulation, six normal cells were selected for tracking close to the initial location of the EC-A cell, and also at a similar distance from, but on the other side, of the wound. A similar number of EC-N cells were also tracked for all control simulations. Analysis of these results showed that neither in control simulations, or in wound simulations where tracked EC-N cells were distal from EC-A cells, did EC-N cells undergo cell division. However, in approximately one in five wound simulations, EC-N cells close to the mutated cell divided a limited number of times (maximum two to three divisions). As can be seen by examination of figure 5, this resulted in clonal EC-N sizes which were substantially smaller than those resulting from the EC-A cells under wounding conditions ($p < 0.001$ in every case), and also less than EC-A clone sizes under control (non-wound) conditions. There was no clear correlation between the propensity of an EC-N cell to divide and its distance from the wound, but multiple EC-N cell divisions were more frequently seen when the original cells were located close to an EC-A cell in proximity to a large (750 μm) wound. However, even in these cases, clonal cell numbers resulting from these cells remained substantially smaller than those generated by EC-A cells following wounding.

The location of EC-A and EC-N cells post-wounding was tracked in the model, with the results shown in figure 6. At each time step, the distance of every tracked cell from the centre of the clonal mass (assuming at least one cell division had taken place) was calculated, and the average for all cells plotted. It can be seen that in the case of the smallest wound, the mutated cells were dispersed over an area approximately 50–100 μm in radius, with there being little dependence on the proximity of the original mutated cells to the wound edge. Tracked EC-N cells close to the EC-A cell typically dispersed over a smaller distance of 50 μm or less (error bars are not shown for these cells owing to the small numbers that divided). However, in the case of the larger wounds, there was a substantial increase in the radius of dispersal if the original EC-A cell was located close to the wound edge, with cells actively migrating or being passively pushed 150–200 μm from the centre of the clone. For the EC-N cells that divided post-wounding, most daughter cells were located within 100 μm of the centre of clonal mass, even if the original cell in close proximity to the wound.

Figure 7 shows screenshots of simulations at 50 iteration intervals following the creation of a 500 μm scratch wound at confluence (150 iterations). Results for $d = 3$, 15 and 30 are shown. Closure of the scratch wound typically occurred within 125 iterations. There was no difference in terms of the number of EC-A (green) cells in each of the three cases, but it is clear that in the case where the original mutated cell was located close to the wound edge, the resulting clone was dispersed throughout a much larger area of the monolayer.

3.3. Experiment 3: exploring the effect of removal of a subset of normal cells

Examination of the simulation results obtained following abrogation of a randomly dispersed subpopulation of EC-N cells after 150 iterations of growth showed that there was a high degree of variability in terms of the number

![Graphical representation of growth curves obtained from virtual experiment 2.](http://rsif.royalsocietypublishing.org/Downloaded from http://rsif.royalsocietypublishing.org/)

Figure 5. Growth curves obtained from virtual experiment 2, where scratch wound is created at iteration 150: (a) 250 μm wound, (b) 500 μm wound and (c) 750 μm wound. (Online version in colour.)

revealed $p$-values $< 0.01$ for $d = 3$ and $d = 30$, and $p < 0.05$ for $d = 15$.

In the case of the largest, 750 μm wounds, EC-A cells positioned at $d = 3$ and $d = 15$ from the wound edge consistently expanded, generating numbers of progeny that were statistically significant relative to controls. The mutated cell underwent at least five rounds of division in five of six cases when $d = 3$ ($p < 0.05$), and in all cases when $d = 15$ ($p < 0.01$). However, there was a larger variation in the final number of EC-A cells obtained.

Creating a single cell mutation and wound at lower or higher densities yielded similar behaviour in terms of capacity for the EC-A cell to form a clone of mutated cells (results not shown).
of subsequent cell divisions observed in the mutated EC-A cell (table 1). Growth curves obtained for EC-A cells following the ablation of different fractions of the confluent normal cell population are shown in figure 8a. The large error bars reflect the variance between replicates, with EC-A cells undergoing between zero and five rounds of division.

These results indicate that the likelihood of cells undergoing multiple rounds of division is related to the fraction of EC-N cells removed. For $k = 0.01$, EC-A cells undergo multiple division in only one of six cases. This increases to three of six cases for $k = 0.05$ and $k = 0.1$, and to five or 6/6 for $k = 0.25$ or greater. Similar trends are apparent for the cases where the normal cells were removed and simulation restarted at iteration 125 or 175. No relationship between the proximity of the nearest deleted cell to the EC-A cell and the propensity of the latter to undergo multiple divisions was observed (data not shown).

Figure 6. Spatial dispersal of cells obtained from virtual experiment 2, where scratch wound is created at iteration 150. Mean displacement of cells from centre of clonal mass is plotted 200 iterations post-wounding for (a) 250 μm wound, EC-A cells; (b) 500 μm wound, EC-A cells; (c) 750 μm wound, EC-A cells; (d) 250 μm wound, EC-N cells; (e) 500 μm wound, EC-N cells; and (f) 750 μm wound, EC-N cells. (Online version in colour.)
Examination of the maximum number of divisions of the EC-A cell upon removal of increasing fractions of the normal cell population reveals some interesting results. It can be seen that the removal of 0.1 or 0.25 of normal cells resulted in four or more divisions of the EC-A cell in half of all cases. However, the results show that although removal of larger fractions of EC-N cells results in consistent expansion of the EC-A cell population, ultimately, a smaller total number of EC-A cells are obtained compared with the cases where 0.05–0.25 of the normal population was removed. This result is corroborated by examining the expansion of EC-N cells following the restart of the simulation (figure 8b). This shows that for $k = 0.4$ and 0.6, the EC-N population expands much more rapidly than when smaller numbers of cells are removed.

### 4. Discussion

Unlike mathematically based models of cellular systems, agent-based modelling allows explicit spatial representation of the interactions between individual cells and their influence on population growth. The ability to introduce single or agent subsets, and to elaborate or modify rule sets, provides a means to model how a mutation compromising the formation of stable cell : cell contacts may influence relative growth patterns of normal and abnormal populations within a tissue.

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**Table 1.** Number of rounds of division of single transformed EC-A cell following abrogation of a fixed fraction ($k$) of the confluent cell population at iteration 150. Frequency of number of rounds of divisions observed in $n = 6$ simulations.

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*Figure 7.* Screenshots obtained at 50 iteration intervals during simulations associated with virtual experiment 2 when single cell switched to EC-A and 500 μm scratch wound created at iteration 150. EC-N cells shown in blue and EC-A cells in green. Row (a) EC-A cell initially located $d = 3$ cell radii from wound edge. Row (b) EC-A cell initially located $d = 15$ cell radii from wound edge. Row (c) EC-A cell initially located $d = 30$ cell radii from wound edge. (Online version in colour.)
of EC-A cells. This is due to its position relative to the wound edge, with those located close to the injury showing a highly significant propensity for increased growth relative to controls. This result raises the possibility that depletion of even a small number of cells may be sufficient to provide a permissive microenvironment for the growth of cells carrying nascent mutations, provided that such a cell happens to exist close to the wound site. Furthermore, the presence of mutated cells that multiply and become dispersed over a wide area may enhance the proliferation of normal cells in the vicinity, which supports the results observed in experiment 1. Future model development to improve computational efficiency will allow the investigation of larger wounds, or the identification of threshold distances beyond which a decrease in cell crowding is insufficient to allow cell growth.

In the case of larger 500 or 750 μm wounds, our results suggest that the distance of a randomly mutated cell from the wound edge has less effect on the number of EC-A daughter cells ultimately produced. However, mutated cells placed in closer proximity to the injury were observed to result in an increased spatial dispersal of EC-A progeny. This behaviour can be attributed to the enhanced migration potential of both EC-A and EC-N cells released from physical contact inhibition exerted by the presence of opposing neighbouring cells in the confluent monolayer, and the resulting movement into the wound space, as previously reported in Walker et al. [32]. Lateral spread of single carcinoma cells has been observed clinically within the urinary bladder and has also been shown to have an effect on disease prognosis [33]. Although our current rule set assumes identical migratory behaviour between EC-N and EC-A cells, this is likely to be an over-simplification. Future adaptation of our model to include differences in migratory behaviour between normal and mutated cells will allow us to explore this behaviour further.

Finally, experiment 3 has shown that removal of even a small fraction of normal cells may be sufficient to induce the expansion of a randomly positioned EC-A cell in some cases, with the likelihood of EC-A clonal growth increasing with larger abrogated EC-N populations. However, a complex relationship between the growth potential of the normal and E-cadherin-compromised population appears to exist, depending on the proportion of the normal population that is removed. When a moderate number (10–25%) of EC-N cells are instantaneously removed, EC-A cells undergo up to five successive divisions before confluence is reached. However, if the size of the abrogated EC-N fraction is further increased, the release from contact inhibition of the remaining EC-N population is rapid enough to result in a high growth rate of these cells, which ultimately impedes the expansion of the pre-malignant cells owing to space restrictions. This effect is similar to that observed in the case of initially randomly dispersed EC-A populations in experiment 1, and shown in figure 3, which suggest that when a population of cells are mutated in a growing monolayer, the expansion of normal cells is actually enhanced in comparison with EC-A cells, provided that the latter are dispersed, rather than clustered.

The predictions of our model are dependent on a number of assumptions, including that growth is limited to the plane of the monolayer only, and also that the E-cadherin-compromised cells are subjected to the same space limitations as normal cells. When there is no room for these cells to grow

Figure 8. Growth curves obtained from virtual experiment 3, where subpopulation of EC-N cells ablated and single cell mutated at iteration 150. (a) Total number of EC-A cells. (b) Total number of EC-N cells. (Online version in colour.)
or divide in the plane of the monolayer, they become quiescent in the same way as their normal counterparts. Obviously, these assumptions may not be valid for a three-dimensional in vivo tissue structure, where out-of-plane cell division is possible. Extension of our model to three-dimensions would allow future consideration of these alternative scenarios. Additionally, we can extend this model to explore the effect of including additional changes in behaviour that may be associated with mutated cells—for instance, enhanced active cell migration.

We have neglected explicit representation of any intercellular signalling mechanisms in this version of the model. It is possible that pre-malignant cells may express membrane-bound or soluble factors that may influence growth of local EC-A or EC-N cells. Our model incorporates an abstraction of a previously investigated mode of EGFR-mediated juxta-crine signalling, which requires contact with at least one other cell during the G1 phase to permit cell cycle progression (see Walker et al. [30] for further discussion). Future experimental identification of relevant intercellular signalling mechanisms would allow inclusion within future versions of the model, using either an abstracted or true multiscale approach (e.g. as described in Walker et al. [34]).

In spite of these limitations, our computational explorations have revealed non-intuitive emergent patterns of behaviour when heterogeneous populations are subjected to microenvironmental perturbations representing tissue injury or damage. Future experimental testing and iteration will enable the model to be refined to further explore how normal tissue homeostasis may positively and negatively influence the process of tumour initiation and spread.

5. Conclusions

We have described how an agent-based model of epithelial cells can be used to explore the emergent behaviour of a heterogeneous monolayer of cells consisting of normal epithelial cells and a variable subset of cells that have lost the ability to form stable contacts via homotypic E-cadherin interface.

Our results suggest that the ability of one or more EC-A cells to proliferate is governed not only by their number, but also by their microenvironment, specifically, their direct contact with other EC-A or normal cells, or the proximity to any disturbance of confluence, such as a wound.

Although simplistic and currently restricted to representing two-dimensional interactions, we have shown that our model is capable of making non-intuitive predictions that provide a realistic basis for experimental testing in vitro.

References

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