Simulating psoriasis by altering transit amplifying cells

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Received on September 26, 2006; revised on February 11, 2007; accepted on February 4, 2007

Associate Editor: Satoru Miyano

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ABSTRACT

Computational models of tissue homeostasis will facilitate a deeper understanding of many diseases. They link molecular networks, cellular differentiation and the spatial and temporal organization of tissues. Here we show an approach which is able to computationally turn a healthy \textit{in silico} epidermis into one with four central properties of psoriatic epidermis. We achieve this by altering a single simulation parameter in the cellular differentiation program of the simulated epidermal keratinocytes: the fractional time period during which transit amplifying cells proliferate (\(r\)). Prolonging \(r\) results in the four main pathological characteristics of psoriatic skin: (1) an absolute increase of the germinative compartment, (2) an absolute increase of the differentiated compartment, (3) a higher proportion of germinative cells and (4) a marked reduction in turnover time. The prolongation of \(r\) is able to increase the proliferation capacity of the epidermal tissue without altering the cell cycle frequency.

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1 INTRODUCTION

Systems biological models are expected to become important frameworks for integrating and validating hypotheses concerning epidermal homeostasis. Here we present an application for our computational epidermis model for the modeling of a psoriasiform epidermis.

Psoriasis is a chronic disease of unsolved pathogenesis affecting skin and joints in 1–3% of the general population. It is characterized by inflamed and scaly skin lesions and has a great impact on the patient’s quality of life. The skin lesions show hyperproliferation and altered differentiation of epidermal keratinocytes, marked infiltrates of T cells and neutrophils, and a distinct increase in skin papillae. Although different loci have been identified by genome-wide scans the cause of psoriasis is still unknown. A functional contribution of T cells in the etiology of psoriasis is strongly inferred from the presence of T cells in lesioned skin and the beneficial response to immunosuppressive drugs. However, recent results indicate that T cells are perhaps not essential in inducing the chemokine/cytokine profile seen in psoriasis (Gudjonsson and Elder, 2006). This motivates for searching functional alterations of keratinocytes. Jun proteins (c-Jun, JunB and JunD), together with the Fos, ATF and CREB proteins are main components of the activator protein 1 (AP-1) transcription factor. The balance of Jun proteins determines whether cells progress through the cell cycle. In psoriasis, JunB is downregulated throughout the tissue, while c-Jun is upregulated in the lower regions of the epidermis. Using an epidermis-specific double knock-out mouse model, it was shown that the epidermis-specific down-regulation of JunB induces cytokine/chemokines, which are known to recruit inflammatory cells, thereby contributing to the establishment of the clinical and molecular features observed in psoriasis and psoriatic arthritis (Zenz et al. 2005). Nevertheless, as the mRNA level of JunB is reported to be unchanged in psoriasis, the cause of the expression change of JunB and c-Jun proteins remains unclear.

Furthermore, it remains unclear during which states of differentiation keratinocytes may be altered. As hyperproliferation is a hallmark of psoriasis, stem cells and transit amplifying (TA) cells which form the main part of the proliferating cells in the epidermis, can be expected to be functionally altered. This is corroborated by studying the spatial distribution of c-Jun which reveals that in psoriasis c-Jun is strongly expressed in the lower proliferation compartment of the epidermal tissue while there is hardly any expression in healthy epidermis (Mehic et al., 2005). This proliferation compartment is mainly composed of stem cells and TA cells, capable of prolonged tissue regeneration \textit{in vivo} (Li et al., 2004). In healthy skin, K15 is a marker of stem and TA cells within the basal layer, which is expressed at the deep ridges of adult human skin (Webb et al., 2004). In psoriasis, this marker is downregulated due to immunological activation of the keratinocytes (Waseem et al., 1999). Although this means that K15 cannot be used as a marker of stem cells and TA cells in psoriasis, it supports the hypothesis that functional alterations in the proliferative compartment of keratinocytes can be found. This hypothesis is enforced by findings of altered mRNA levels of several markers of differentiation and proliferation in TA cells (Fransen et al., 2005). Taken together, the given observations point to an inherent malfunction in the behavior of TA cells.

Surprisingly, no reduction in the cell cycle time can be observed in psoriasis, although an increase in the number of cell divisions has been described (Casteljns et al., 2000). A possible solution to the question, of how the proliferation capacity of TA cells could be increased without altering the cell cycle time, is altering the time during which TA cells proliferate. This could be due to a lack of differentiation of TA cells which keeps them...
prolonged in a state of proliferation as it is indicated by the observed imbalance of the proteins c-Jun and JunB which partly control differentiation and proliferation.

To summarize, we set out to determine the effect of the time fraction during which TA cells proliferate on epidermal morphology. For this we use our computational simulation of epidermal tissue (Grabe and Neuber, 2005). To the knowledge of the authors, currently no computational simulation of a psoriatic morphology has been described yet. While Iizuka (Iizuka et al., 1996) modeled the static size of psoriatic rete ridges, to our knowledge, Heenen et al. (Heenen et al., 1987) developed the first and up till now only mathematical model of cell renewal in psoriatic epidermis on the basis of epidermal compartment sizes. Their model describes the kinetic changes in epidermal homeostasis leading to psoriasiform morphology. We use the works of Heenen et al. as a baseline and compare our morphological simulation results to their numerical results.

In our simulation, which is a strong simplification of the complex in vivo situation, the epidermis’ proliferation compartment is composed of stem cells and TA cells. Stem cells are modeled with an unlimited proliferation capacity and an unlimited age while TA cells have a standard maximal age, common to all keratinocytes. In our simulation, TA cells are only able to proliferate a limited fraction ($\tau$) of their maximal lifetime before they become differentiated cells and unable to proliferate. We altered this keratinocyte differentiation scheme in silico towards a prolonged proliferation time period of TA cells. Our results demonstrate that indeed this prolongation of proliferation time of TA cells leads to the central characteristics of psoriasiform morphology described earlier (Heenen et al., 1987).

### 2 METHODS

Our model consists of only one cell type, the keratinocyte. For each cell a distinct qualitative differentiation stage is assumed: stem cell, TA cell, early or late stratum spinosum cell and granular cell. Additionally, cells have a defined age (unlimited for stem cells), a cell cycle and concentrations of molecules in their environment. A detailed description of the model can be found in (Grabe and Neuber, 2005). The parameters have been set on the basis of physiological data (Bauer et al., 2001; Castelijns et al., 1998; Heenen et al., 1998; Hoult and Leaky, 2003). The simulation is structured in hours simulation time. During each cell cycle (62 h), a stem cell creates a TA cell and a TA cell produces an early stratum spinosum cell.

This proliferation only occurs, when enough space for proliferation is available. All cells, except stem cells, have a maximum life span (1000 h) after which they are removed from the simulation. TA cells may proliferate during a first fraction of their lifetime denoted $\tau$ (8%). This value is based on our previously published model (Grabe and Neuber, 2005). After this period, TA cells become differentiated stratum spinosum cells.

The general morphology of the simulated epidermis is determined by its basal lamina which is forming two rete ridges of non-elastic shape. We used our in silico epidermis model to estimate the effects of prolonging $\tau$ from 8 to 100% while keeping the absolute life span for all cells in the simulation at the same, constant value. From $t=0$ to $t=2000$ h, the simulation is initially populated with cells. The system then develops with $\tau=8\%$ in a steady state until $t=4000$ h. During this time statistical data representing a healthy epidermis are collected. Then $\tau$ is manually increased from 8 to 100%. After equilibration of the system average simulation data of the diseased epidermal state are collected for the time period $t=6000$ to $t=8000$ h.

### 3 RESULTS

#### 3.1 Impact on tissue morphology

Figure 1 shows the effects of prolonging $\tau$. The morphology of the in silico epidermis after its initial population with cells is stable from $t=2000$ h onwards (Fig. 1a). At $t=4000$ h $\tau$ is raised to 100%, while keeping the absolute life-time constant. The morphology of the epidermis changes rapidly. At $t=6000$ h in the new equilibrium, the epidermal thickness is considerably increased, while the stratum granulosum is partially disturbed (Fig. 1b). This is a result of the increase of stratum spinosum cells. Although neither the stem cell nor the TA proliferation frequency have been changed, a psoriasiform morphology can be observed.

#### 3.2 Impact on tissue differentiation

As can be seen in Figure 2a, increasing $\tau$ from 8 to 100% has considerable impact on tissue differentiation. Except in the instable period of tissue perturbation from 4000 to 6000 h, the mean age remains constant throughout the simulation. The total cell count rose by 50% from a mean of 366 cells in the time period from $t=2000$ to $t=4000$ h up to a mean of 549 cells from $t=6000$ to $t=8000$ h. This rise suggests that the increase in cell number leads to the thickening of the epithelial compartment observed in psoriasis. A quantitative comparison of the changes in tissue differentiation induced by increasing $\tau$ can be found in Table 1. The absolute number of proliferating cells in the tissue increased by 155%, while the number of differentiating cells increased by 41%. The proportion of proliferating cells in the tissue (proliferation index),

Fig. 1. Effects of prolongation of $\tau$ on epidermal morphology. Simulated epidermal morphology at (a) $t=4000$ h; (b) $t=8000$ h.
representing the germinative compartment of the epidermis, rose by 73%, while the differentiating compartment decreased by 6%. The strongest increase was observed in the number of TA cells (+410%). Stratum granulosum cells decreased by 1%.

### Table 1. Changes of central simulation properties induced by increasing the proliferation time fraction of TA cells

<table>
<thead>
<tr>
<th>Property</th>
<th>Healthy</th>
<th>τ = 100% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of stem cells (SC)</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Number of TA cells</td>
<td>11</td>
<td>56</td>
</tr>
<tr>
<td>Number of early spinosum cells</td>
<td>132</td>
<td>222</td>
</tr>
<tr>
<td>Number of late spinosum cells</td>
<td>141</td>
<td>191</td>
</tr>
<tr>
<td>Number of granulosum cells</td>
<td>64</td>
<td>63</td>
</tr>
<tr>
<td>Total number of cells</td>
<td>366</td>
<td>549</td>
</tr>
<tr>
<td>Number of proliferating cells (TA+SC)</td>
<td>29</td>
<td>74</td>
</tr>
<tr>
<td>Number of differentiating cells</td>
<td>337</td>
<td>476</td>
</tr>
<tr>
<td>Proportion of proliferating cells</td>
<td>8%</td>
<td>13%</td>
</tr>
<tr>
<td>Proportion of differentiating cells</td>
<td>92%</td>
<td>86%</td>
</tr>
<tr>
<td>Turnover time</td>
<td>759 h</td>
<td>450 h</td>
</tr>
<tr>
<td>Growth fraction</td>
<td>18%</td>
<td>40%</td>
</tr>
</tbody>
</table>

3.3 Impact on tissue kinetics

In order to assess the consequences of an increase of τ in a quantitative manner, we also collected time-averaged kinetic values for healthy epidermis (τ = 8%) in the time period from t = 0 to t = 4000 h and for the potential psoriasiform epidermis (τ = 100%) in the time period from t = 6000 to t = 8000 h (Fig. 2b and Table 1). The growth fraction, representing the fraction of proliferating basal cells, increased from 18 to 40%. This increase stands for a thickening of the germinative layers in psoriasis. Furthermore, the mean turnover time reduced from 759 to 450 h, thereby reflecting the disturbance in maturation which is characteristic for psoriasis.

4 DISCUSSION

The qualitative features of psoriatic epidermis have been described as (1) an absolute increase of the germinative compartment, (2) as an absolute increase of the differentiated compartment, (3) as a higher proportion of germinative cells, and (4) as a marked reduction in turnover (Heeneman and Galand, 1984). As summarized in Table 1, (1) after an increase of τ, our simulation resulted in a marked increase (+155%) in the number of proliferating cells, and (2) as a marked increase (+41%) in the differentiated compartment. (3) The observed increase (+73%) in the proportion of proliferating cells while the relative size of the differentiated compartment was reduced (–6%) fulfills the third criterion. (4) Accordingly, the growth fraction, denoting the fraction of proliferating cells in the germinative layers of the epidermis, rose from 20 to 40%. The fourth criterion is fulfilled by a markedly decreased (–41%) turnover time. From these results one may conclude that the four hallmarks of an in situ morphology of psoriatic lesions can be observed after prolonging τ. It is known that also some differentiated keratinocytes have a principal proliferative capacity (Li et al., 2004). This fact is omitted in the simulation. Instead it is assumed that the in silico TA cells are representatives of all non-stem-cell proliferative cells. Members of this non-stem-cell-compartment of epidermal tissue have a rather short lifetime, limiting their proliferation power.

Our results show that enhancing the proliferation time of these keratinocytes indeed produces a psoriasiform epidermis which is also consistent with the observed unchanged proliferation cell cycle time in psoriasis. Histologically, psoriatic epidermis can be divided into two types: one with a prominent granular layer (G-plus) and one without a granular layer (G-minus). Chronic and resolving psoriatic lesions exhibit a granular layer (Iizuka et al., 1996). After prolonging τ the simulation reacted with a markedly thickened epidermis, which still expressed a stratum granulosum. This resembles the physiological situation of resolving psoriasis (G-plus). The expression of the stratum granulosum depends on the differentiation capability of the keratinocytes. Therefore, after inhibiting the calcium influx, the simulation system can also generate a G-minus psoriatic epidermis (not shown). The total number of cells in psoriatic epidermis is reported to be increased by four to five times compared with normal epidermis (Iizuka et al., 1996), although this depends on severity. We observed a limited increase because our simulation currently does not allow...
the extension of rete ridge which limits the increase of cell numbers observable with our simulation.

Experimental evidence indicating altered TA cells in psoriasis was given in the introduction. A cause for this alteration cannot be given today. Interestingly, several indications of an altered calcium signaling have been reported in psoriatic epidermis which is a main driver of keratinocytes differentiation. Therefore, an altered Ca\textsuperscript{2+} signaling could be related to the balance of differentiation and proliferation inside non-stem cells towards a prolonged proliferative activity. Indications of the involvement of Ca\textsuperscript{2+} signaling in psoriasis are manifold. The intake of calcium channel blockers (nifedipine, felodipine and amlodipine) which are directed against voltage-gated Ca\textsuperscript{2+} channels is associated with outbreaks of psoriasis after a latent period (McKenzie et al., 2003). Oda et al. showed in animal experiments that the calcium sensing receptor (CaR), if genetically inactivated, leads to skin similar to those in psoriasis. With the advent of biomarkers like integrin \( \beta \)-4 and CD71 psoriatic TA cells might be studied accordingly (Li et al., 2003). Oda et al. observed that keratinocyte cultures derived from lesional and non-lesional psoriatic skin could not mobilize their intracellular calcium stores and that capacitative influx of extracellular calcium was defective (Karvonen et al., 2000). Calcium influx also occurs via cyclic guanosine monophosphate-gated channels. McKenzie et al. reported that in psoriatic keratinocytes, a non-functional splice-variant of a cGMP-gated Ca\textsuperscript{2+} channel is higher expressed and that high expression of this splice-variant leads to a loss of protein expression for the functional cGMP-gated Ca\textsuperscript{2+} channel (McKenzie et al., 2003).

5 CONCLUSION

To our knowledge, there is currently no computational morphological model of psoriasis. As an initial step towards such a model, we described recent findings concerning the role of Jun proteins in literature pointing towards a possible alteration of the function of TA cells. As TA cells have a limited time during which they are actively proliferating, a prolongation of this time period presents an alternative reason for hyperproliferation opposite to the reduction of cell cycle times, which has been ruled out (Casteljns et al., 2000). We used our computational model of human epidermal homeostasis (Grabe and Neuber, 2005) to estimate consequences of changes in the active proliferation lifetime fraction of TA cells while keeping the overall lifetime of all cells, except stem cells which have an unlimited lifetime in the simulation, constant. Our results fulfill the four pathologically defined qualitative criteria described in the literature. As a cause for the prolonged proliferation time fraction, the role of Ca\textsuperscript{2+} signaling was discussed. Further experimental investigations are necessary to elucidate the role of TA cells in psoriasis. It can be concluded that the results presented here motivate an investigation of the role of TA cells in psoriasis. With the advent of biomarkers like integrin \( \alpha \)-6 and CD71 psoriatic TA cells might be studied accordingly (Li et al., 2004). Especially a study involving Jun proteins and Ca\textsuperscript{2+} signaling in the context of TA cells seems motivated.

From the viewpoint of bioinformatics, our simulation provides a novel approach for evaluating hypotheses about differentiation-related diseases like psoriasis. Clearly, at the present stage our model is still at the beginning but we were able to demonstrate that computationally modeling epidermal tissue homeostasis on the cellular level is a valuable approach for studying complex homeostatic processes in silico. Models like the one we presented will in future include more information about intracellular biochemical processes which will make them more realistic and informative. We expect that in silico tissue models will enable a much more detailed understanding of the complex interacting processes in epidermal biology with a multitude of applications.

ACKNOWLEDGEMENTS

We thank Dr Minne van der Haak and Prof Dr Thomas Wetter for proofreading.

Conflict of Interest: none declared.

REFERENCES


