Morphology of melanocytic lesions *in situ*: Supplementary

T. Balois and M. Ben Amar

Laboratoire de Physique Statistique, Ecole Normale Supérieure, UPMC Univ Paris 06, Université Paris Diderot, CNRS, 24 rue Lhomond, 75005 Paris, France
Institut Universitaire de Cancérologie, Faculté de médecine, Université Pierre et Marie Curie-Paris 6, 91 boulevard de l'hôpital, 75013 Paris, France

Some details of the article require more explanations. First, we will come back on the biological context seen in sec.2. especially the micro-environment, then we will describe more precisely the model in sec.3. and the range of parameters we used.

1 Biology of the skin

Here we will focus on the interactions between cells in the normal skin, then in a tumor.

1.1 Micro-environment in normal skin

The epidermis needs a more thorough description. First of all, keratinocytes and melanocytes are not the only cells found in the epidermis. A normal epidermis also exhibits Langherans cells and Merkel cells. Langherans cells ($\sim$ 2% of epidermis cells [1]) are immune system cells. They detect antigenes which penetrate the epidermis and present them to lymphocytes. The Merkel cells are pressure sensing cells. As those cells are scattered, they are not taken into account here.

The micro-environment of a cell is very important for its fate. For instance melanocytes cultivated without keratinocytes display some characteristics of melanoma cells that do not appear in their presence

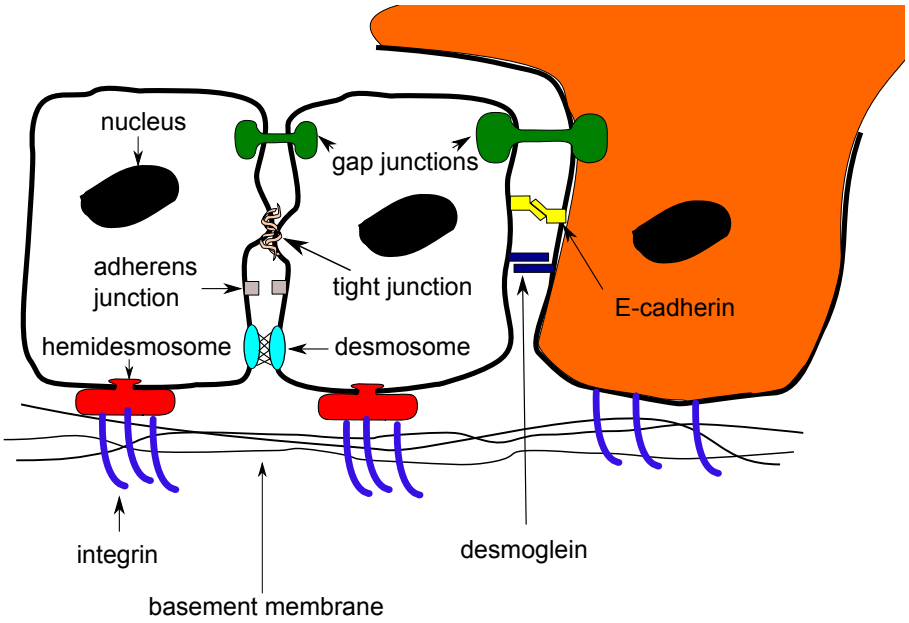

Figure1. Schematic representation of the adhesion mechanisms between healthy cells. On the left, in white keratinocytes, and on the right in brown melanocytes.

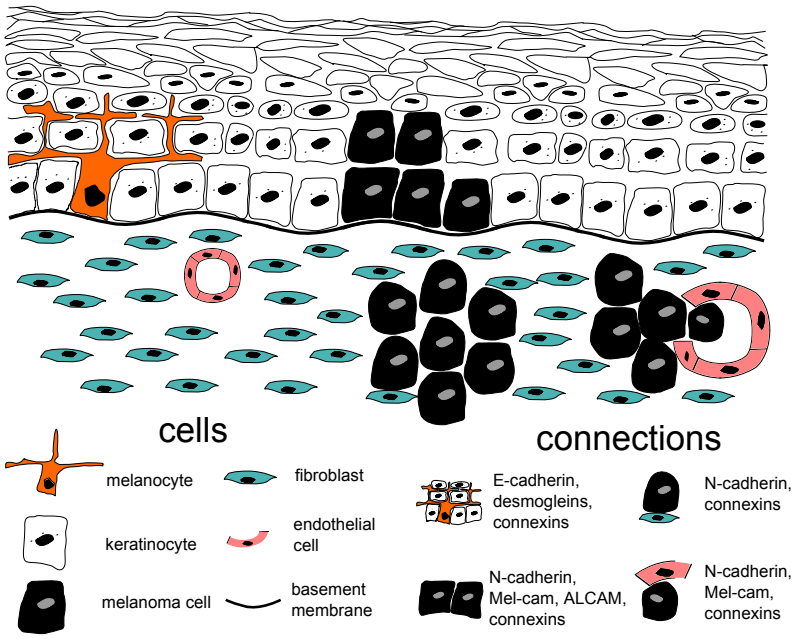

Figure2. Representation of the skin with several type lesions adapted from [2]. The malignancy increase from left to right, with a loss of keratinocytes interactions and an increase of interactions between melanoma cells.

[2]. Therefore, the mechanisms of interactions between cells and their environment are of utmost importance (see Fig.1). Keratinocytes are strongly bound to the basement membrane through hemidesmosomes joining the intracellular keratin network to the collagen fibres of the basement membrane through integrins. Keratinocytes are linked by several types of junctions: desmosomes, tight junctions, adherens junctions and gap junctions (see Fig.1). Tight junctions are mainly constituted of occludins, proteins binding the membranes of neighbour cells. Desmosomes are connected through cadherins and link the keratin network of the keratinocytes. Adherens junctions are also made of cadherins linking the actin network. Gap junctions are communication channels allowing diffusion of small molecules. Melanocytes binds to the basement membrane through integrins linking the actine network of the cytoplasm to the basement membrane. In healthy skin melanocytes do not bind to each other, but they are strongly attached to keratinocytes through E-cadherins and desmogleins, and communicate through gap junctions with keratinocytes.

1.2 Micro-environment involved in tumors

During melanogenesis, the nature of the bounds change, this process is known as cadherin switch. Interactions with keratinocytes decrease via a diminution of E-cadherins, desmogleins and gap junctions, while the interactions with other melanoma cells increase via an augmentation of N-cadherins, melanoma cell adhesion proteins (Mel-CAM), and activated leukocyte (cell adhesion molecule ALCAM) [2,3]. Growth of melanoma cells relies less and less on the micro-environment, they evade control from the other cells. The new adhesion molecules (N-cadherins, Mel-CAM and integrins) allow a better interaction with fibroblasts and the other endothelial cells, and therefore the invasion of the dermis.

2 Model

First, we describe the equations for nutrients and explain the derivation of the 2D description. Secondly, we will focus on the method used to ascertain the velocity of the cancerous phase $\boldsymbol{v_c}$.

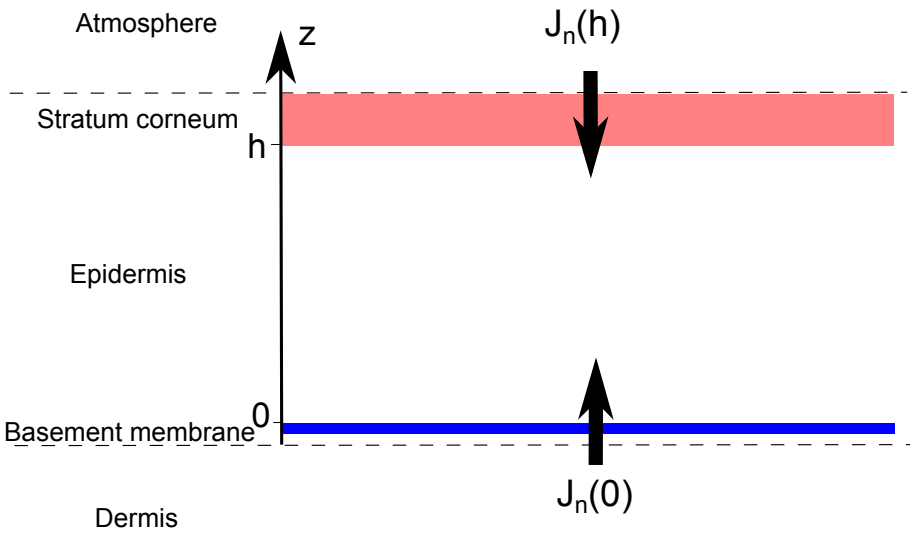

Figure3. Schematic representation of the epidermis.

2.1 Nutrients

Regardless of the kind of nutrients considered (oxygen or sugar), they are small molecules which diffuse in the epidermis while being consumed by the cells so the concentration n is given by:

$$\frac{\partial n}{\partial t} + \boldsymbol{\nabla}.(n\boldsymbol{v}) = \boldsymbol{\nabla}.(D_n \boldsymbol{\nabla} n) + \Gamma_n(\phi, n)$$

where $\boldsymbol{\nabla}$ is the differential operator: $\boldsymbol{\nabla} = (\partial_x, \partial_y, \partial_z)$, $\Gamma_n(\phi, n)$ represents the nutrient consumption. Considering the velocities involved (growth speed $\sim$ 0.3mm per day), the left term of the equation is negligible, so the nutrients are at diffusion equilibrium. Averaging along the z-axis, we get:

$$0 = \boldsymbol{\nabla}_{//}.(D_n^{//} \boldsymbol{\nabla}_{//} < n >) + < \Gamma_n(\phi, n) > + \frac{1}{h} \left[J_n(h) + J_n(0) \right]_0^h$$

where $\boldsymbol{\nabla}_{//} = (\partial_x, \partial_y)$ is the differential vector in the plane of the epidermis, $< n >= h^{-1} \int n dz$, $< \Gamma_n(\phi, n) >= h^{-1} \int \Gamma_n(\phi, n) dz$, $J_n(h)$ and $J_n(0)$ are the nutrients flux in $z = h$ and $z = 0$ (see Fig.3).

We assume a simple form for nutrient flux:

$$J_n = \alpha(n^0 - < n >) \text{ , so } \frac{1}{h} \left[J_n(h) + J_n(0) \right]_0^h = S_n(n_s - < n >)$$

where α and n^0 depend on the position and on the nutrient (oxygen mostly diffuses from the stratum corneum [12], whereas the sugar comes obviously from the dermis). Because the epidermis is hypoxic and for simplicity, the nutrient consumption is taken linear in both ϕ and n:

$$< \Gamma_n(\phi, n) >= -\delta_n \phi_c < n > -\kappa(1 - \phi_c) < n >$$

Droping the average symbols <> and the plan symbol //, we get the governing equation for nutrients in 2D:

$$0 = D_n \Delta n - \delta_n \phi_c n - \kappa(1 - \phi_c) n + S_n(n_s - n) \tag{1}$$

2.2 Mechanical and thermodynamical aspects

The mass balance equation can be written for the cancerous phase, ϕ_c, and the healthy phase ϕ_h:

$$\begin{cases} \partial_t \phi_c + \boldsymbol{\nabla}.(\phi_c \boldsymbol{v}_c) = \Gamma(\phi_c, n) \\ \partial_t \phi_h + \boldsymbol{\nabla}.(\phi_h \boldsymbol{v}_h) = \Gamma(\phi_h, n) \end{cases} \tag{2}$$

In order to determine the velocity of the cancerous phase $\boldsymbol{v_c}$, we apply a variational principle. First, we take a simple form for the free energy $\mathcal{F}_s$:

$$\mathcal{F}s = \int \left[\Psi(\phi_c) + \epsilon^2 (\boldsymbol{\nabla}\phi_c)^2\right] dxdy$$

We add a viscous friction W between phases of the form:

$$W = \int_\Omega \frac{M\phi_c}{2} \left(\boldsymbol{v}_c - \boldsymbol{v}_h\right)^2 d\Omega$$

So the variation of energy per time unit $\mathcal{Q}$ called the Rayleighian [4] is written as:

$$\mathcal{Q} = \int_\Omega [\underbrace{\frac{M\phi_c}{2}\left(\boldsymbol{v}_c - \boldsymbol{v}_h\right)^2}_{\text{dissipation}} + \underbrace{\left(\frac{\partial \psi}{\partial \phi_c} - \epsilon^2 \Delta\phi_c\right)\left(-\boldsymbol{\nabla}\cdot(\phi_c \boldsymbol{v}_c) + \Gamma_c\right)}_{\text{variation of free energy}} - \underbrace{p\nabla\cdot(\phi_c \boldsymbol{v}_c + \phi_h \boldsymbol{v}_h)}_{\text{incompressibility}}] d\Omega$$

where p is a Lagrange multiplier interpreted as the pressure that insure the incompressibility condition. Minimizing $\mathcal{Q}$ by a variationnal analysis with respect to both velocities ($\boldsymbol{v}_c$, $\boldsymbol{v}_h$) gives us a new set of equations, that can be interpreted as the force equilibrium for each phase:

$$\begin{aligned} & M\phi_c\left(\boldsymbol{v}_h - \boldsymbol{v}_c\right) + \phi_h \boldsymbol{\nabla} p = 0 \\ & \underbrace{M\phi_c\left(\boldsymbol{v}_c - \boldsymbol{v}_h\right)}_{\text{friction}} + \underbrace{\phi_c \boldsymbol{\nabla}\left(\frac{\partial \psi}{\partial \phi_c} - \epsilon^2 \Delta\phi_c\right)}_{\text{stress of the cellular phase}} + \underbrace{\phi_c \boldsymbol{\nabla} p}_{\text{hydrostatic pressure}} = 0 \end{aligned}$$

Eliminating the pressure, gives us the relative motion between the two phases:

$$\boldsymbol{v}_c - \boldsymbol{v}_h = -\frac{(1-\phi_c)}{M}\boldsymbol{\nabla}\left(\frac{\partial \psi}{\partial \phi_c} - \epsilon^2 \Delta\phi_c\right)$$

Considering that the mass center is not moving, $\phi_c \boldsymbol{v_c} + \phi_h \boldsymbol{v_h} = 0$, is a particular solution for an incompressible flow. This allows to write a Darcy-like equation:

$$\boldsymbol{v_c} = -\frac{(1-\phi_c)^2}{M}\boldsymbol{\nabla}\left(\frac{\partial \psi}{\partial \phi_c} - \epsilon^2 \Delta\phi_c\right)$$

Therefore, we have $\Sigma = \frac{\partial \psi}{\partial \phi_c} - \epsilon^2 \Delta\phi_c$ and $K = M^{-1}$, the same expression described in sec.3.2. for the cancerous phase:

$$\partial_t \phi_c + \boldsymbol{\nabla}.(-K\phi_c(1-\phi_c)^2 \boldsymbol{\nabla}(f(\phi_c) - \epsilon^2 \Delta\phi_c) = \gamma\phi_c n - \delta\phi_c \tag{3}$$

where $f(\phi_c) = \frac{\partial \psi}{\partial \phi_c}$ and takes the form described in sec.3.2. Eq. 1 and 3 depends on several parameters that needs to be evaluated.

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