Forces & Cancer

From Biology to Physics, from the Laboratory to the Patient

Summary

CHAPTER 1: THE ADCHIEVMENT

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CHAPTER 1: THE ADCHIEVMENTS

Introduction to Physical Oncology (PO)

The study of mechanical signals in oncology has undergone a spectacular revival since the late 1990s.

The causes are many:

- The exhaustion of the model based on genetics and molecular biology, and the question is cruel when one thinks of disappointed hopes: 'How many cancer patients have been cured by targeted therapies?' [1]
- The development of cell culture techniques in 3 dimensions (3D), closer to living tissue than 2D cultures.
- The inclusion of the extracellular matrix as an essential actor in carcinogenesis and response to treatments.

These last two points coincide to impose new models, in vitro or in vivo, to study carcinogenesis and the impact of treatments that take into account what is cancer: 'Solid Tumors' - as opposed to hematological malignant diseases - are organs composed of a tumor tissue itself surrounded by another tissue, the ExtraCellular Matrix (ECM) often called Stroma[2].

[1] Basically, Chronic Myeloid Leukaemias have not been cured but have seen their lives prolonged for so long that one can equate this with a cure. In "solid tumors", patients with breast cancer treated with adjuvant trastuzumab when the tumor overexpresses HER2, see their percentage of recurrence divided by two. This represents 3% of breast cancers. That's all. In order to be exhaustive, it is necessary to emphasize the minimal part of Targeted Therapies for the "chronicisation" of some cancers such as the breast or the prostate.

[2] ECM or stroma, both terms are equivalent. We also find 'Conjunctive Tissue'.

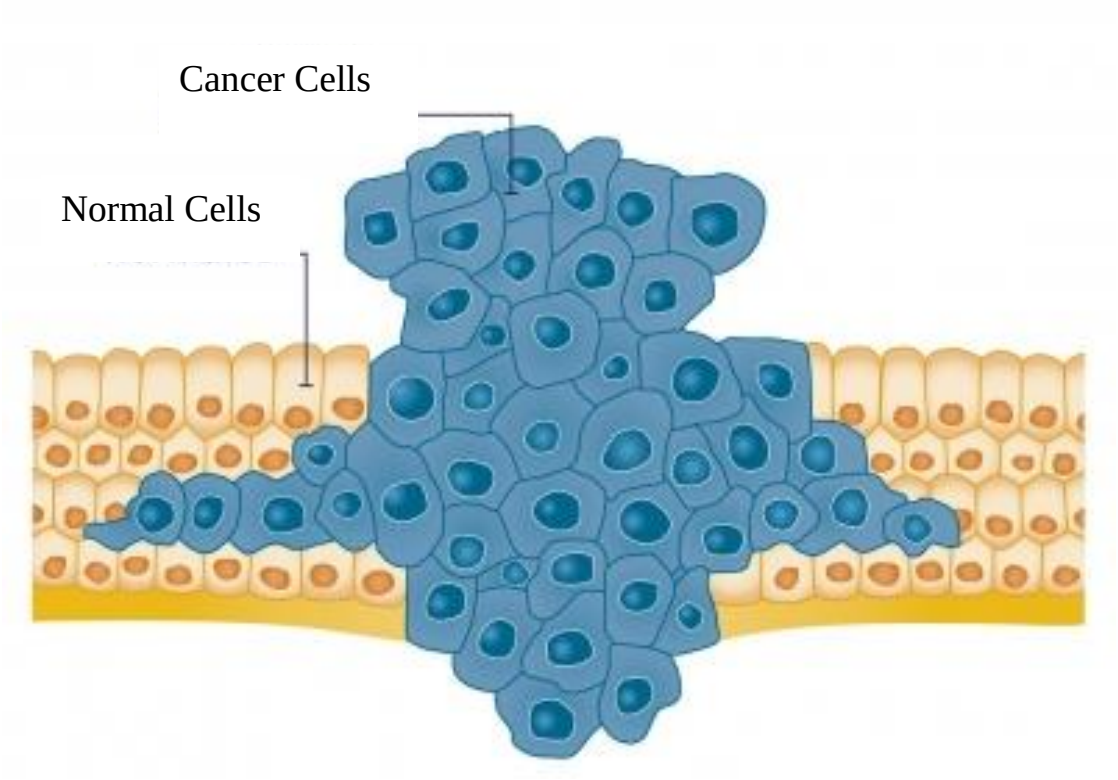


Diagram showing a tumor invading healthy tissues

Figure 1

This pattern shows an 'epithelium' that is the only tissue of cancer origin that we will take into account here and which represents more than 90% of the solid tumors.

Normal cells are of Euclidean geometry as well as normal tissue.

The medical definition of cancer is the crossing of a barrier, the basal membrane here below.

The tumor cells and the tissue they form have a different geometry, fractal, less familiar to our mental representations, hence the description by histologists when they describe cancers of a disorder, anarchy, whereas this geometry is perfectly organized and analyzable.

Beneath the basal membrane is the stroma that contains the vessels that will nourish and oxygenate the epithelium.

By reaching this stroma the cancer will quickly constitute an autonomous ECM around him, with his own vessels (neovessels) to nourish and to oxygenate. This tumor vascularization is called neovascularization.

Cancer then becomes autonomous, histologically by constituting an 'organ-cancer', and metabolically by developing an energy machinery of its own.

In the following we shall consider that the addition of the basement membrane and the stroma below are of the same nature as the ECM, and we shall not distinguish between the two.

Metastases are generated by cancer cells that leave the primary tumor and go to different organs to constitute other ‘organs-cancer’ at a distance.

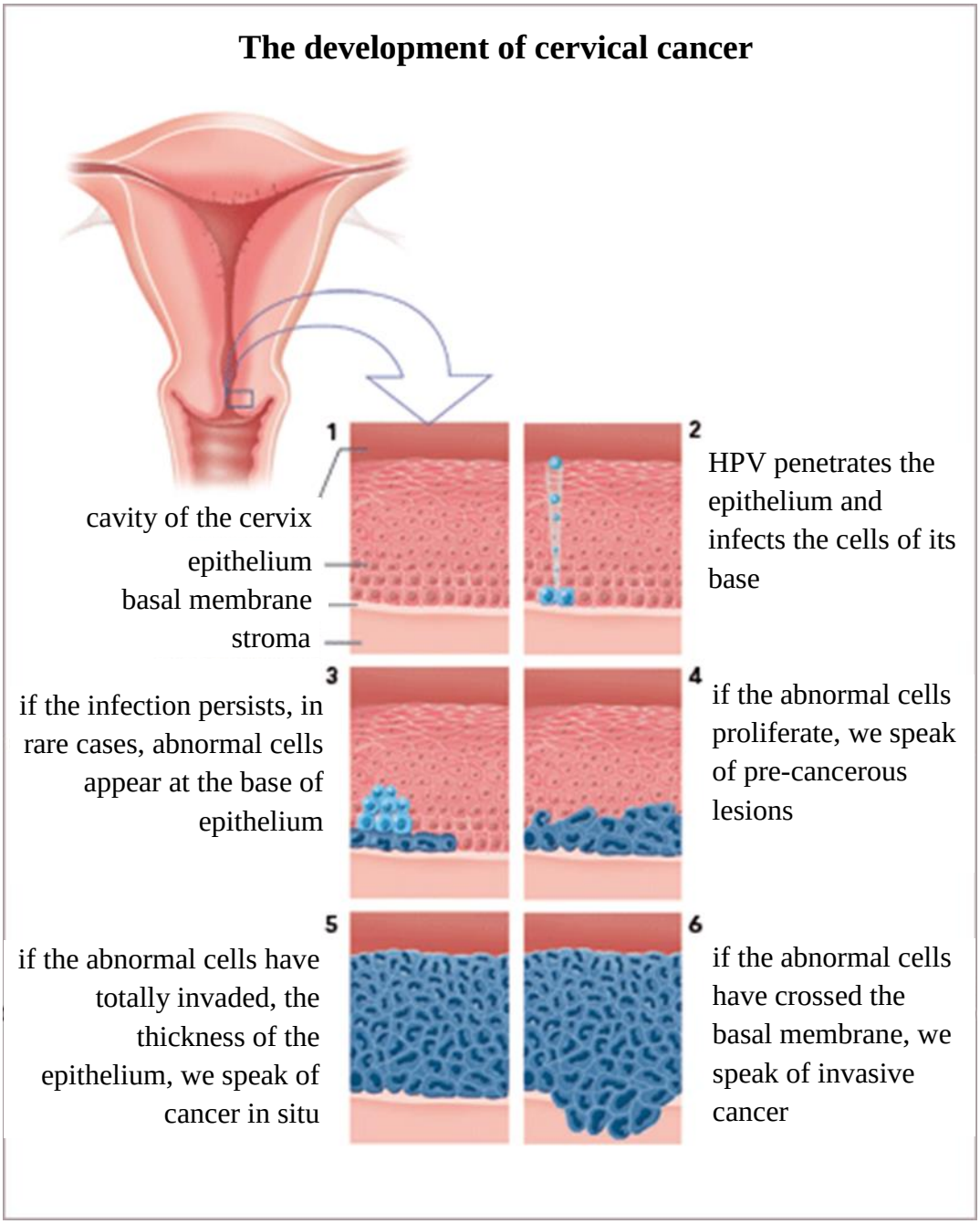


Figure 2

The stages of cancerogenesis in the case of cervical cancer.

Progressions of the same type are known in cancers of the colon, bronchus, and in many covering epithelia. Pre-cancerous conditions are also found in many glandular epithelia such as the breast or prostate.

Cancer is formed over a long time and is for a long time reversible.

The example above shows the very gradual transition over a number of years of a benign anomaly of the cervical epithelium caused by a viral infection (HPV or

Human Papilloma Virus): mild dysplasia. Most often it disappears as well as the next step, severe dysplasia. But the longer the dysplasia persists and increases, the less reversible it is.

It can then lead to 'cancer in situ' which is not a cancer in the medical sense (no crossing of the basal membrane) but has all the other characteristics (except the ECM).

The Time Scale

In response to questions from patients 'but for how long?' Doctors have learned to respond, 'for several years' and ‘for many years’ when the disease follows the most common sequence: dysplasia with or without metaplasia, in situ, primary tumor then metastasis.

Let us take the example of the increase in the rigidity of the ECM of a normal epithelium, whether due to inflammation or some other cause. At this slow modification of its environment the cell will respond by a rapid elastic deformation which will accompany the increase of rigidity. Then it will adapt to maintain its architectural integrity by generating an attraction force that will counteract the action of the ECM. This over a longer time. And then one day the geometric distortions will reach a sufficient intensity so that the mitotic spindle until then strictly parallel to the basal membrane ends up exceeding a threshold and lead to asymmetric cell division with loss of polarity. Then the cell divisions will stack cells forming a fractal tissue.

The Stages of Cancer

For very practical reasons we talk about Stages III to describe cancers that are 'Locally Advanced' which can still be treated to be cured. But for this it will require medical treatment before the surgery (and / or radiotherapy). If this medical treatment is not sufficient, then they join the IV - those who are metastatic from the start or after relapse- and which we do not know how to cure: we then enter the palliative treatments to obtain -at the best- a complete remission with disappearance of all the signs or a control that is expected to be as long as possible on the growth of the tumor.

The Place of the Physical Oncology

Cancerology BioMechanics owes its recent progression to the application to cancerology of "Physical Oncology" (PO) as a new way of studying cancer.

PO is defined as the study of physical and more specifically mechanical signals in the functioning of cancer from dysplasia, in situ, to metastatic diffusion, and therefore throughout the spectrum of carcinogenesis.

In its still most active part, in vitro or ex vivo, the PO has no pretention to have a therapeutic role. On the other hand, his recent orientation towards in vivo forces us to think his role as a possible treatment and the choice of pancreatic cancer as the first target corresponds to a patent medical ‘unmet need’.

A system throughout the body

Thus, we see an organic entity - the ECM - that sends mechanical information to all the tissues of all organs, such as a decentralized nervous system that would send mechanical and not electrical pulses, thus ensuring coordination and control of behavior between all the cells / tissues of the organs and the organism. The cells are in charge of distinguishing the mechanical noise (including that related to the gravity) of the signals of change of the environment. They must also distinguish the variable or periodic signals from the constants, the outputs being different.

In the phenomenon of carcinogenesis, it is logical to assume that the predominant action is that of constant or quasi-constant signals such as a slow increase in the rigidity and slow transformations of the fibrillar composition of the ECM rather than as a stored accumulation of transient signals.

In PO, cancer is no longer merely a "disordered" genetic and biological machinery, but also and may be the result of a progressive change in the architecture of the tissue, from the normal Euclidean to the cancerous fractal. And it is this change in the distribution of forces, pressures, constraints ... in cancer that will modify the functioning of the genetic and molecular machinery through the conformational changes imposed on DNA (and epigenetics) by these forces transmitted to the nucleus from outside the cell.

An Emerging Science, a Specific Scale

PO is an emerging science at the interface of mechanics and oncology, and therefore of physics and biology. The biological and physical signals present in cancerous tissues as well as in normal tissues are very different. And, although they constantly interfere with each other, the laws that govern them are disjointed.

Table 1

Difference between biological and physical signals

	Support	Transmission of the signal	Modulation of the signal	Nature of the Signal	Action	Reversibility in space
Biological	Liquid Incompressible	Slow Step by step	Difficult	Biochemical	Slow	No Unidirectional
Physical	Semisolid (20% of cell volume) Compressible	Fast Almost instantaneous	Easy	Mechanical	Fast	Yes Bidirectional

The PO studies the impact of mechanical signals on the appearance of the cancer phenotype. The relevant phenotype in PO is that of cells and tissues. Nevertheless, the tissue phenotype is more complex than the cellular phenotype. Thus, by tissue phenotype we will hear the phenotype - cellular - differentiation, growth, apoptosis, cell death and ability to migrate to which we will add tissue architecture.

And it is impossible here not to quote Mina Bissell: "in oncology the tissue phenotype is dominant in relation to the cellular genotype".

The mechanical signals spontaneously present in the tissues or used in PO to modify the cellular or tissue phenotype are made of forces, pressures directed towards the inside of the cell (pull) or outwards (push) or tangential (shear) forces, forces at the ECM / tissue interface and cell / cell interfaces. The generalization leads us to speak of the application of a field of constraint (or better of a tensor

field). It can be seen that the PO is located on the mesoscopic scale, that of the cells and the tissues. The evolution has produced a hierarchy of structures to distribute the mechanical signals according to the scale of the structure in question, such as nested Russian dolls: on the mesoscopic scale, the pressures involved are of the order of a few to a few hundred or thousands of Pascal (Pa).

Genetics and molecular biology are at the scale of molecules and genes, therefore microscopic.

Oncology and its imaging are on a macroscopic scale, except for the microscopic diagnosis on the biopsy.

Finally, a point must be recalled which conditions any ambitious approach of the PO: we know how to cure localized cancers. We do not know how to cure metastatic cancers, with a few exceptions such as testicular cancers. Locally advanced cancers are a real challenge. And, we should not forget, the vast majority of cures are obtained through surgery and / or radiotherapy that have a long past behind them.

The role of half-century drugs like hormone/chemotherapy is to increase the cure rate by eliminating tumor cells already in metastatic position. It is therefore a probabilistic treatment that improves by a few percent the number of cures. It is still marginal if we talk about healing. And it will remain so.

But they play a fundamental role in the 'chronicisation' of certain cancers, including hormone-dependent cancer such as breast and prostate cancer.

Targeted therapies play a much smaller role. That of immunotherapies is expected to be much more useful but cures will remain rare except tumors already known to be manipulable immunologically speaking like melanoma and kidney.

The PO should also be situated between biology (molecular biology, genetics, immunology) and mechanics, as shown in Table 2 below.

Table 2

	Input Signal	**Output Signal**
Biology	Biological	Biological
Mechanobiology	Mechanical	Biological
Physical Oncology	Mechanical	Mechanical

In the process of recognizing this new branch of biology, the PO has undoubtedly benefited from the very mechanistic approach of the appearance of metastases, as illustrated by the number of articles on the "journey of the metastatic cell" which must change all its physical parameters from the primitive tumor, to cross many mechanical barriers before arriving in the organ where it will find a favorable ground to form a metastasis. But we will not dwell on this aspect of PO which leads more to diagnostic developments on the circulating cells than to therapeutic developments. The entanglement between mechanical and biological signals is part of the functioning of life, but it can be seen that only the PO is studied independently of the laws of biology.

The achievements of PO research

Cellular rigidity

The PO first progressed by the direct approach to the measurement of the intrinsic mechanical properties of the cell: the systematic determination of Young's Modulus - or modulus of elasticity - of normal cells, benign tumor cells and malignant tumors cells and tissues of the microenvironment of tumors, in fact the ECM in its various components including tumor neoangiogenesis. The in vitro or liquid approach to isolated cells has recently been supplemented by ex vivo studies on biopsies.

Cellular / tissue PO: looking for physical parameters

It is on this scale that research is still the most active, whether it is the isolated cell, the culture in 2 Dimension (2D) (less and less) or the 3D culture.

It was first necessary to make assumptions based on the analysis of the results of the tensioning of the intracellular components.

The study of the impact of the applied forces of which the CytoSKeleton (CSK) to cells has based on several techniques including micropatterning. A cell is fixed on a support whose rigidity (stiffness) and adhesion surface can be varied. The support can be very rigid as in plastic culture dishes (traction > 10,000 Pa) or can be modulated from a few Pa to several thousands of Pa as with media of the "gel" type. This allowed us to understand how the physical environment - here the traction exerted by the only adherent surface - modifies the CSK, and the cellular phenotype. The "traction force microscopy" and especially the Atomic Force Microscope (AFM) are used to measure the stiffness, the forces involved.

Micropatterning was first applied to only one or a few cells. It is 3D which has allowed us to scale to the equivalent of tissue organized in space and to focus on the cell and tissue globally without taking into account the underlying biochemical and genetic mechanisms. But the study of supramolecular complexes such as the organization of integrins in junction points and structures of cellular organization such as the CSK and its components - microtubules, actin filaments and intermediate filaments - have been necessary steps in the understanding of maintenance or name of the architectural integrity of the cell and tissue and allowed to imagine hypotheses as to carcinogenesis.

Cellular micropatterning also taught us a lot about the organization of the CSK when the stresses applied to one face of the cell were varied and showed the transmission of mechanical signals from the environment of the cell to the nucleus, Through the CSK. But the limitation to one or a few cells and the unidimensional character of the application of the constraints on a single face, has long limited the practical interest.

For growth-dependent 'anchorage-dependent' cells, division assumes an increase in the CSK tension that is obtained when the cell spans on a rigid support, equivalent to a very rigid ECM.

Conversely, to decrease the rigidity of the ECM to the point of not being able to compensate for the cellular traction, leads the cell to differentiation or apoptosis and stops the division.

The mechanoreceptors

The best example are integrins.

Mechanotransduction is the term used to describe all cellular / tissue responses to forces imposed from the outside and passing through mechanoreceptors.

But this term must remain neutral and not systematically imply a biological response, such as a cascade to the nucleus, a transduction.

It is the realization of the very artificial nature of the culture conditions in 'Petri dishes' made of glass or plastic, which led to the taking into account of the role of the very important rigidities of these supports. Of course, the rigid, flat and large supports allowed to stimulate the culture of the normal and cancer cells in vitro outside their environment; This environment - the ECM and the neighboring cells organized in space - will impose the organization in the space of the tissues, the polarity of the cells and their phenotype, all this by the mechanical signals.

The Young's Modulus in vitro

Measurements have shown that normal cells are more rigid (about 1.2 kPa) than their malignant counterparts (about 0.8 kPa) and that the ECM is even more rigid (about 2.5 kPa).

When a doctor, or a patient, feels a "lump in the breast", what is hard is the ECM. The tumor, inside, is less hard than the normal mammary gland.

Two-dimensional cell cultures do not reflect the in vivo situation and are very poor predictors of the effect of cancer treatments. This is the main reason why NCI launched a 3D in vitro cancer research program in 2009. The NCI Physical Sciences in Oncology site associates 12 universities in the US with the goal of having physicists and oncologists working together, people who do not speak the same language.

Numerous cell lines, normal, transformed or cancerous were measured. The Young's modulus of cancer cells is of the same order of magnitude as a little less than 1 kPa and the normal / cancer ratio is around 1.5. The correlation between the decrease in rigidity and therefore the increase in deformability and the metastatic potential is very strong.

It was thus possible to obtain prognostic information or to try to use liquid biopsies to measure the rigidity of circulating cancer cells, again with a very strong correlation between the decrease in rigidity and the prognosis.

It is important to emphasize how the PO is returning to a unicist conception of cancer: there is ultimately little physical quantitative variation between different cancers. Contrary to the genetic-molecular approach, treatments using the PO approach are likely not to be 'personalized'.

In all cases the methodology used must be taken into account, with absolute values varying between the use of the Atomic Force Microscope, the optical tweezers, the microfluidics...

Ex vivo

Some studies have measured the tissue rigidities on biopsies, ie pieces of tumor taken, kept alive and analyzed by Atomic Force Microscopy. The most representative study is that of M Plodinec (2011), which confirms the measurements obtained on cells in vitro in 2 and 3 dimensions.

AFM measurement of Young's modulus on human breast cancer biopsies reveals a signature specific to each cancer stage. Comparing healthy tissue with cancerous tissue from a benign tumor, the Young's modulus distribution (representative of elasticity) is between 1.9 kPa and 3.7 kPa for the benign tumor and between 1.1 KPa and 1.8 kPa for healthy tissue. Tissues from more invasive pre-metastatic tumors show a dominant peak between 0.3 and 0.8 kPa. For metastasis, the cell must have a certain degree of flexibility and deformability enabling it to move in its environment, thus its Young's modulus is lower than that of healthy cells.

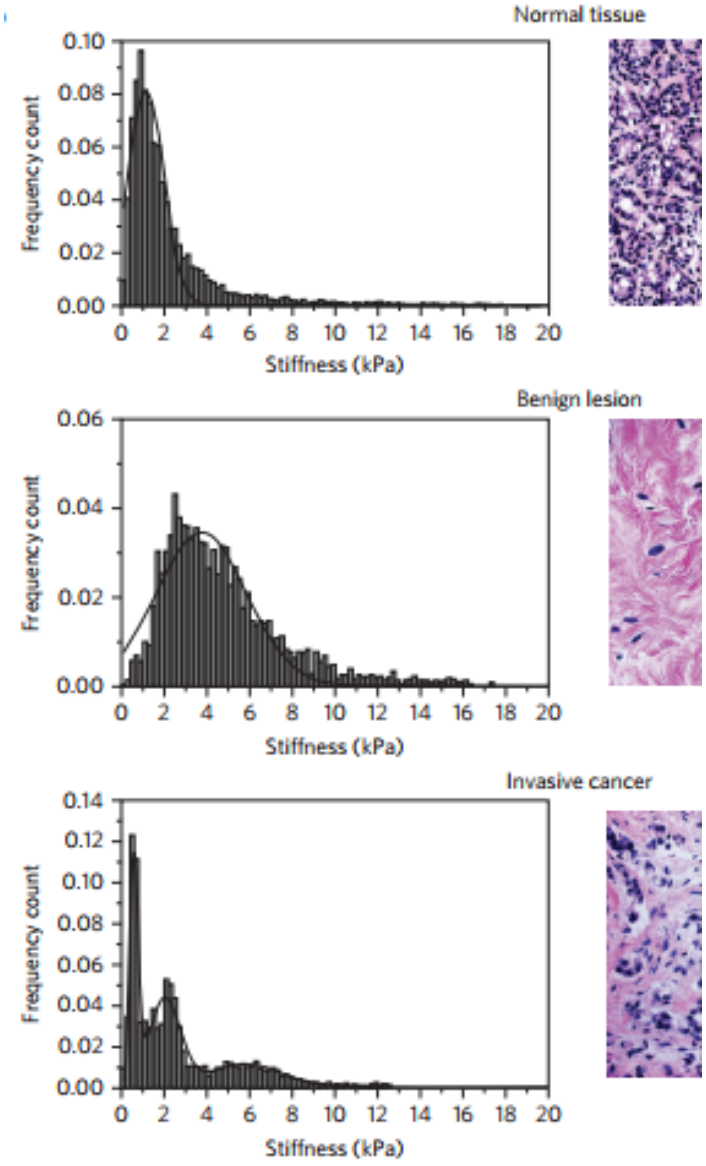


Figure 3

A sample of the tissue stiffness values present in breast biopsies (M Plodinec et al)

Cellular response

Finally, experiments were carried out to quantify the responses of the cells, for example in 3D culture, to mechanical stimulations. There is the 'pull' of the cells at the interface with the ECM, the tensions at the intercellular interface and the intracellular forces generated by the CSK.

The first cultures on 3D began in 1989 and matured in 1992. This organization in space between the cells makes it possible to retain the mechanical and biological intercellular links as they are present in vivo. But in the case of the study of the

cancerous phenomenon, it is representative only of the cancerous tissue itself: the organ organization associating the ECM surrounding the tumor tissue is absent. More precisely, the culture medium around the 3D will be able to be a virtual ECM from which signals are made to act. For example, Matthew Paszek in 2005 applies a constraint to a 3D culture of human cancer cells by varying the collagen concentration: it induces the variation in the surface tension applied to the cells in culture. It starts from a normal breast acinus and will make it evolve towards a cancerous breast tissue using exclusively variations of surface tension.

This article is the first to have highlighted the central role of the rigidity of the ECM as a factor of carcinogenesis and its maintenance in the evolution of the disease. It also shows that increased CSK tension disrupts the 'polarity' of mammary cells, activates their multiplication, and distorts the central, spherical lumen.

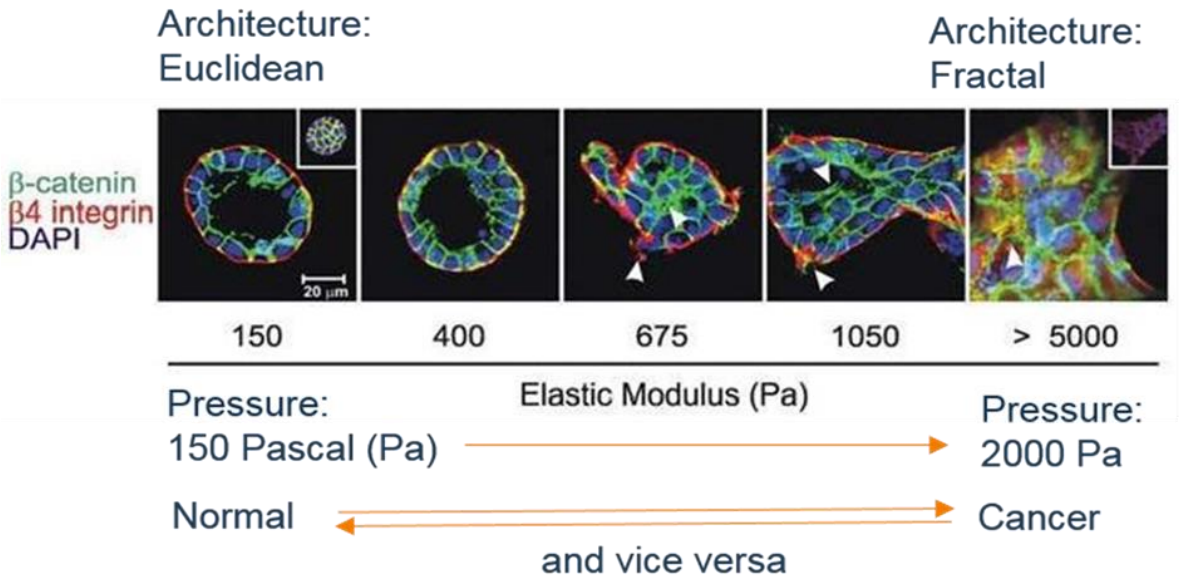


Figure 4

Tumor stiffness in patients or in animal models is the sum of several factors: increased interstitial pressure, tumor volume expansion, neovascularization plays a role as well as fibrosis of the ECM and other less important factors.

But in this experiment alone the rigidity of the culture medium surrounding the acinus varies. From a concentration of 2 g / ml to 4 g / ml, the stiffness measured in Pa by the Young's modulus changes from (on average) 330 Pa to 1590 Pa. Changes in Young's modulus of normal mammary glandular tissue (167 Pa),

which increases its modulus from 167 to more than 2,000 Pa in proportion to the amount of collagen added and thus to the rigidity surrounding the breast tissue.

Table 3

From M Paszek's article showing the different Young Modulus values of different mammary tissues.

Tissue or Material	Elastic Modulus (Pa)
Normal Mammary Gland	167 ± 31
Average Tumor (Ras, Myc, Her2/Neu)	4049 ± 938 **
Stroma Attached to Tumor (Ras, Her2/Neu)	918 ± 269 **
Reconstituted Basement Membrane	175 ± 37
Collagen (2.0 mg/ml)	328 ± 87
Collagen (4.0 mg/ml)	1589 ± 380
Plastic (polystyrene) (Callister et al., 2000)	2.78×10^9
Glass (soda-lime) (Callister et al., 2000)	69×10^9

** Mean ± SEM, $p<0.01$

In fact, without saying so, Paszek shows us the transition from Euclidean architecture, that of a normal epithelium, to a fractal architecture, that of cancers.

The desire to find a molecular biology support for these changes makes the article difficult to read for a non-specialist and, above all, obscures the main result: in 3D, the only relevant model, the compliance of the matrix is the main variable to change the cell phenotype appreciated here by cancer markers derived from molecular biology as well as the architectural type of the tissue, thus in total the tissue phenotype. But if this change in geometry is concomitant with accompanying biological changes and is in accordance with what is known of the changes in the molecular biology that accompany the cancerogenesis is the reversibility of the phenomenon of cancerogenesis. Cancer reversion by mechanical signals becomes an objective of the PO.

Similarly, a somewhat forgotten hypothesis of cause of carcinogenesis becomes again topical: the balance between the rigidity of the matrix (exogenous force)

and the tension of the CSK (endogenous force) can be disturbed by prolonged local 'irritation' like chronic inflammation and promote malignant transformation.

Other authors have found similar results in other tumor models and with other mechanical signals.

Let us quote the publication of F Montel et al in 2014.

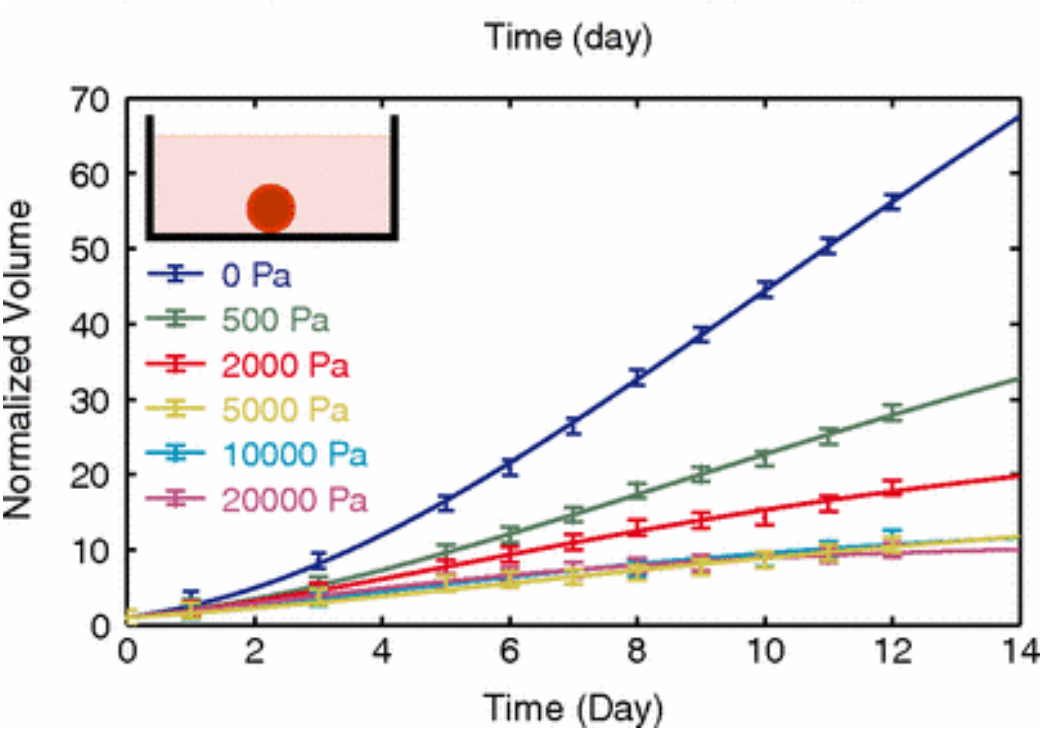


Figure 5

We see here the linear relationship between the growth of the studied tumor (a spheroid) and the applied pressure.

Convergence with Mechanobiology

In mechanobiology, let us mention the work of Emmanuel Farge.

In 2003, he worked on the embryo of the Drosophila fly, a favorite tool of embryologists.

He then showed how a pressure turns into a genetic and molecular signal. Let us sum up his demonstration of 2003. The constitution of a digestive system is very precisely ‘programmed’ in this embryo: it appears in a well-defined place and at a definite moment of the development of the embryo. By applying a simple pressure to another place and at another time he succeeded in 'passing over' the

genetic programming and inducing the formation of a digestive tract at another place and at another time. How is it possible? Because a pressure exerted on a protein, beta catenin, allows it to detach itself from the wall of the cell where it ensures the solidity of the intercellular junctions, and to migrate towards the nucleus of the cell, where it triggers the activation of the Twist gene which will make the twist molecule which is a molecule that transforms little-differentiated embryonic cells into digestive tract cells, which are recognizable and 'fixed' in this digestive function.

This was the first time in embryology that the action of a mechanical (not biological) signal on a gene was shown.

In ‘La Recherche’ he proposes a good popularization of an article published in 2015 in Nature.

The headline of the article summarizes its purpose: ‘The growth of a tumor compresses the adjacent healthy cells and can make them cancerous’. This mechanism involves a protein known in embryology.

We are thus in the framework of MechanoBiology, that is to say the impact of a mechanical signal - a pressure - when translated into a biological message, a molecule.

It is therefore not 'Physical Oncology' that studies the translation of a mechanical signal into a mechanical signal.

But the article by E Farge obliges the Physical Oncologists not only to concern themselves with the mechanical relations between the cells of a cancerous tissue and the mechanical relations between this cancerous tissue and the ExtraCellular Matrix which surrounds it. We must also take into account in the 'balance of forces' the mechanical relations between the ECM and the normal tissues surrounding the tumor.

The continuous pressure exerted by a tumor which proliferates in the colon transforms the neighboring healthy cells into cancerous cells.

We can thus see a propagation - or a creation of tumor at a distance - that obviously concerns not only the colon but all the cancers for which catenin beta is important.

Moreover, this paper provides quantified data that verifies the little information we have on the 'Young’s Modulus' of tissues, ie the measure of their rigidity. And

we find data similar to those described on breast cancer biopsies by M Plodinec in 2011, or about 1.2 kPa for the pressure exerted by the cancerous tissue.

In Vivo

We will end with an approach that takes into account all these advances and has a direct therapeutic ambition: the application of a field of constraint to a tumor in vivo.

Mechanical signals used in in vitro models - surface tension, osmotic pressure, gravity - are not usable in vivo.

Curiously, in vivo, though the only way to arrive at a therapeutic proposal, is very little present in the PO literature.

It is nevertheless the major step, that which makes it possible to envisage the passage of laboratory object to potential therapeutic tool.

In PO, we find only the article by R Brossel et al (Plos One, 21 April 2016) which shows the possibility of acting on a tumor grafted subcutaneously in the rodent by applying a constraint.

In this perspective, the 'field of constraint' becomes the therapeutic agent.

This stress is exerted via ferric nanoparticles, therefore magnetizable, subjected from outside the animal to a magnetic field gradient. The nanoparticles then act as (Bio)Actuators transforming a part of the magnetic energy into mechanical energy.

It was first necessary to make assumptions based on the analysis of the results of the tensioning of the intracellular components.

Feasibility

In 2014, an in vivo feasibility is carried out on mice grafted by human cancer cells (MDA MB 231) mixed with ferric nanoparticles: the ferric nanoparticles (in black) are distributed around the tumor (as shown in the figure below) due to the very large difference in free surface energy between the particles and the cells.

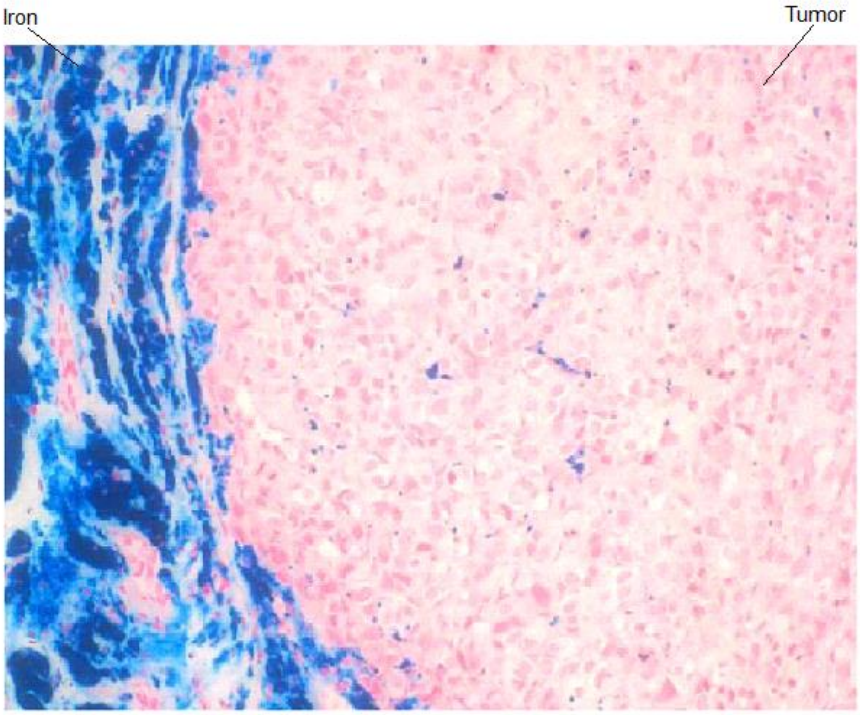


Figure 6

Iron Nanoparticles are used as "(bio)actuators": when a magnetic field gradient is applied to the nanoparticle / tumor assembly, they transform part of the magnetic energy into mechanical energy and create a localized stress field in the tumor.

Thus, the possibility of applying a "field of constraint" (which can be approximated as a first intention to forces and pressures or, by extending the concept, to a tensorial field) to an in vivo tissue can be seen. This involves positioning magnetizable nanoparticles around the tumors and then applying a magnetic field gradient to the tumor / nanoparticle assembly from the outside. It has been known for more than 10 years that a pressure applied to an in vitro tumor, if a sufficient "stress field" is applied, results in a modification of the tissue phenotype (M Paszek, 2005). The innovation concerns the transition from in vitro 3D to in vivo, that is to say from the "test tube" to the animal.

The Proof of Concept in vivo was published in 2016. The aim was to compare a treated group of mice, ie with nanoparticles and magnetic field gradient with three control groups. Figs. 7 and 8 show the experimental set up and the generated forces.

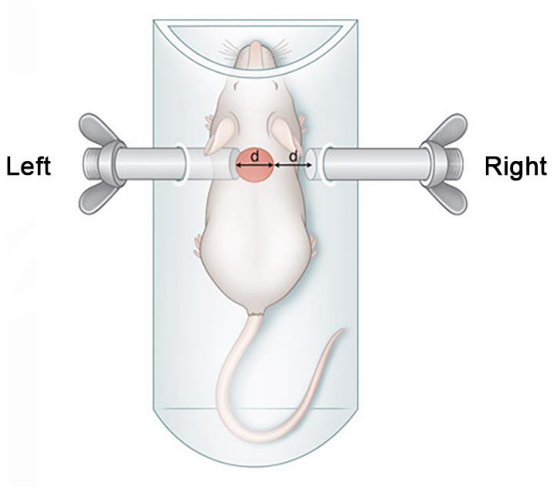


Figure 7

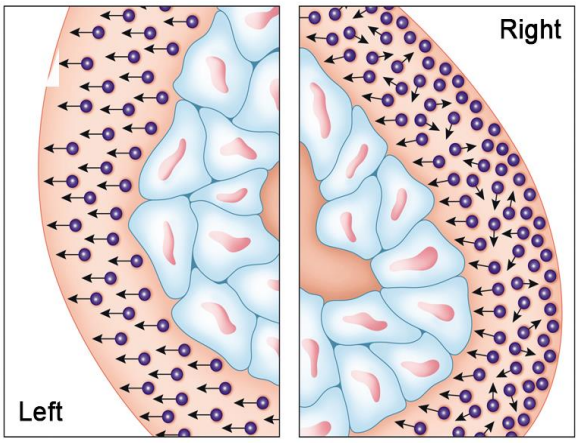


Figure 8

The concept is validated. There was a significant difference between the treated group and the control groups. This difference is related to the volume of the tumor measured in vivo which is significantly decreased (p = 0.015) in the treated group compared to the 3 control groups (with gradient and gradient, gradient and particle free, gradient and particle free) There is also a significant difference in favor of the treated group when measuring the surface of the living tumor ex vivo on digitized histological sections (p = 0.001).

Table 4

Results

Groups of grafted mice	**Median Tumor Volume (mm^3)**	**p (Significance of the difference)**
Groups treated	529	p = 0.015
Control groups*	1 334	
Mean (mm^2)	**Tumor area on histological sections digitized ex vivo**	**p**
Treated Group	7.7	p = 0.001
Control Groups *	23.1 ; 21.4 ; 26,8	

* Three groups of mice: with particles only; with gradient alone; without particles or gradient

The Constraint Field as a Therapeutic Agent

This field imposed from the ECM is superimposed on the one already present in the tumor tissue. Note the difference with in vitro: there is no confinement by the ECM in the in vitro model.

The possible parameters of this stress field relate to intensity and frequency. In the article cited, the estimated intensity is of the order of 10 to 20 Pa for a gradient of the order of 5 T / cm. We were at the physical limits of the method.

The gradient was constant.

This proof of concept used permanent magnets (NeFeB). An electromagnet will be required for an orthotopic pancreatic graft in rodents, and superconductors are the only sources of magnetic field gradient compatible with the thickness of a patient.

The superconducting magnets only allow frequencies below 1 kH but can generate gradients of the order of 50 T / cm.

Most importantly, there is a lack of a description of the forces involved in the tumor in situ in vivo in its entirety and on a smaller scale in the semi-solid structures involved. There is no technology allowing this advance which is a priority to make this therapeutic approach finely modulable and promises like cancer reversion.

CHAPTER 2: THE PAST & THE FUTURE

Let us begin by building a bridge between the 19 th and the 21st century.

The Great Ancestors

In his essay "On Growth and Form" D'Arcy W Thompson shows spectacular correlations between the forms taken by organisms and organs and the laws of mechanics. But it does not go beyond this finding and does not propose a hypothesis of cause and effect.

Before him, we will also remember the name J Wolff, who has shown the quantifiable, controllable, specific and reversible relationship between the forces applied to a bone and the development and internal structuring of bone in various species.

Much later G I Bell will show the influence of forces on intercellular dissociation and C Murray (1926) the principle of optimization in the bloodstream.

The 'Zeitgeist', the spirit of the time, since the 1960s is that the key to the understanding of carcinogenesis is to be found on a molecular scale. The discovery of the genetic code based on DNA followed by the description of transcription and then of signaling-transduction forms a coherent whole on the microscopic scale, that of genetics and molecular biology.

A cancer theory - here we are talking about epithelial tumors - must explain carcinogenesis in all its stages from dysplasia to metastasis.

The theory of somatic mutation (somatic cells: all cells except germ cells) is largely dominant and the birthplace of the cancer is the cell. Contradictory experimental facts are not lacking: the carcinogens are not all mutagenic (the hormones...); the target of the carcinogens may be the ECM and not the cell; An ECM exposed to a carcinogen contacted with non-cancerous tissue can lead to cancerization of this tissue, but not the reverse; a cancerous tissue placed in close contact with a normal ECM may become a normal tissue again.

These last two examples further argue for the reality of the possible reversion of the cancer to the non-cancerous.

The War on Cancer

The symbolism was heavy when President Richard Nixon gave this label to a major cancer study plan in 1971.

The hypothesis is then viral: normal cultured cells are 'transformed' (this is a major step towards cancerization) by the Rous sarcoma virus.

But in real life, in real patients, no viruses.

Yet it was acquired:

That a viral genome, with few genes, could 'force' all the genetic machinery of a mammalian cell to function in a cancerous way: decrease in apoptosis, loss of differentiation, increase in cell divisions, etc.

In 1975 a first 'oncogene', src, was discovered: the normal cell carries a gene which once 'activated' by a virus can transform the cell into culture.

A little later the mutagenic chemical carcinogens join the radiations X, UV... in the pool of the triggers of cancer, without intervention of virus. Asbestos that is neither a molecule nor a mutagen will soon be recognized as responsible for many cancers in people exposed to it.

Other oncogens are described, including a first in humans (ras).

But cancerization is and remains 'multistep', multi-stage, resistant to any explanation of the type: 'a single event (a mutation) can lead to cancer'.

The discovery of 'suppressor genes' adds to the confusion.

In 1989, it was demonstrated that the larger the number of somatic mutations of oncogenes and suppressors, the more a human tumor progresses. And the number, nature and order of mutation varies from one tumor to another in the same tissue of origin and tissue to the other. The perplexity was great: each cancer seems to be an evolutionary experience all by itself.

To organize this growing complexity, close to chaos, hallmarks are proposed that will evolve in number and quality over the years.

But the cancerous tumor remains a moving target with subpopulations, permanent genetic and epigenetic rearrangements.

The era of 'omics' and Big Data (finding the right algorithm to organize the mass of data), from personalized medicine (to each tumor its treatment), resembles a leak forward.

Obviously, Data Mining gives useful information: two cancers of the same origin present sets of totally different gene expressions but with common prognostic values. These 'prognostic indicators' make it possible not to impose unnecessary treatments on many patients, which is to the credit of personalized medicine.

Original sin is common: there is around the cancerous tissue another tissue, the ECM, with its inflammatory cells, its immune cells, its neovascularization, its fibers that generate its rigidity.

The relevant unit in cancer theory - at least solid tumors - is the ECM / tissue pair.

There is another flaw: the reductionist approach.

And Now?

45 years later the war was not won. It is even lost: an increase in the number of cancers, not only due to aging, increase in carcinogenic substances and behaviors...

If a new theoretical construct is to emerge, it is obvious that it must integrate the enormous achievements of the last decades: cancers of genetic origin are few but exist. Mutations such as BRCA in the breast and ovary have a considerable influence on tumor behavior in the daily clinic. Mutations/viruses alone can lead to cancer.

Systems biology provides us with useful insights: networks, fields, attractors are words unfamiliar to the ears of biologists or oncologists, but they try to find rules to organize exponentially growing knowledge. For the moment, we remain in the speculative but relevant hypotheses are tested there.

After research will come the development. Today it is necessary to 'Let Know'

Physical Cancerology attracted little attention so far and was reserved for a small circle of academic gentle(wo)men.

In the US, the National Cancer Institute, after having richly endowed twelve of the best American universities in 2009 to work together cancer physicians and bioengineers in an "Office for Physical Sciences and Oncology" research in mechanobiology and physical oncology. It regularly publishes results of experiments carried out in these teams. Curiously the production remains very centered on the biological functioning of the cancer with a predominance of the mechanobiology. This leads to the publication of numerous articles ending with "and thus the pharmaceutical industry will be able to find targets...", which differs from the goal of the PO which should be to promote treatments based on the use of physical consequences of mechanical signals.

In Singapore, an Institute of Mechanobiology welcomes several hundred researchers dedicated to this new approach to cancerology.

Sign encouraging a dedicated magazine just appeared: Convergent Science Physical Oncology.

In Europe, there are a few scattered academic laboratories (France, Germany, Spain...) but the PO is not present in European or national programs or in scientific meetings.

But the pedagogical effort advances: see the references 'Introduction to the PO'

Physical oncology must emerge from the shadows and address the cancer community. We are part of this dynamic.

The continuation of the In Vivo research in the continuity of the Proof of Concept

The in vivo Proof of Efficacy of the action of a stress field will be on the treatment of a human pancreatic cancer grafted into a mouse pancreas.

This technology involves the association of two Medical Devices: a Magnetic Field Gradient Generator, similar in cost, volume... to a current MRI, and a consumable: Nanoparticles that will bring iron, magnetizable, around the tumor.

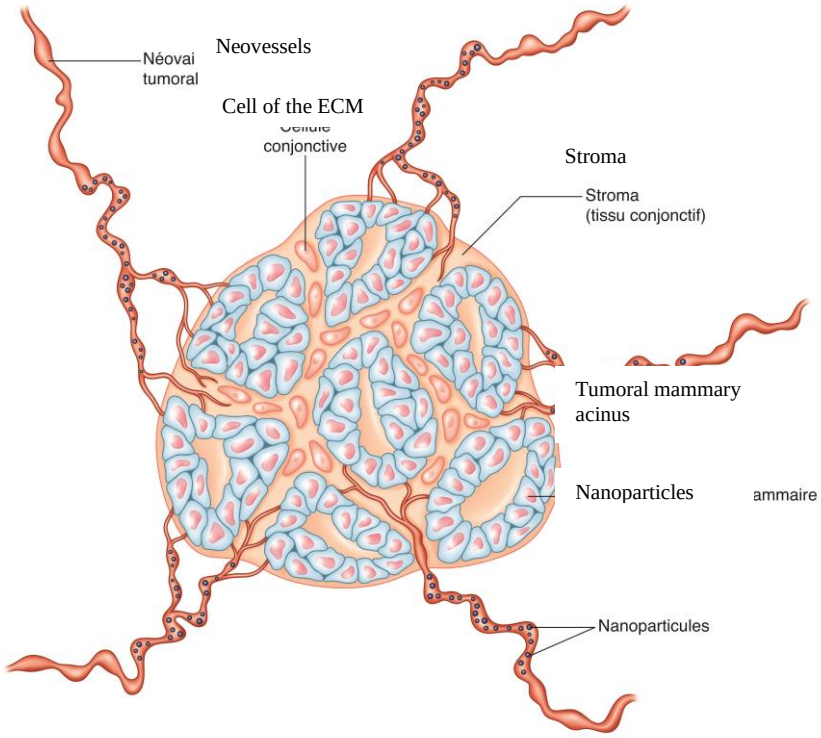


Figure 9

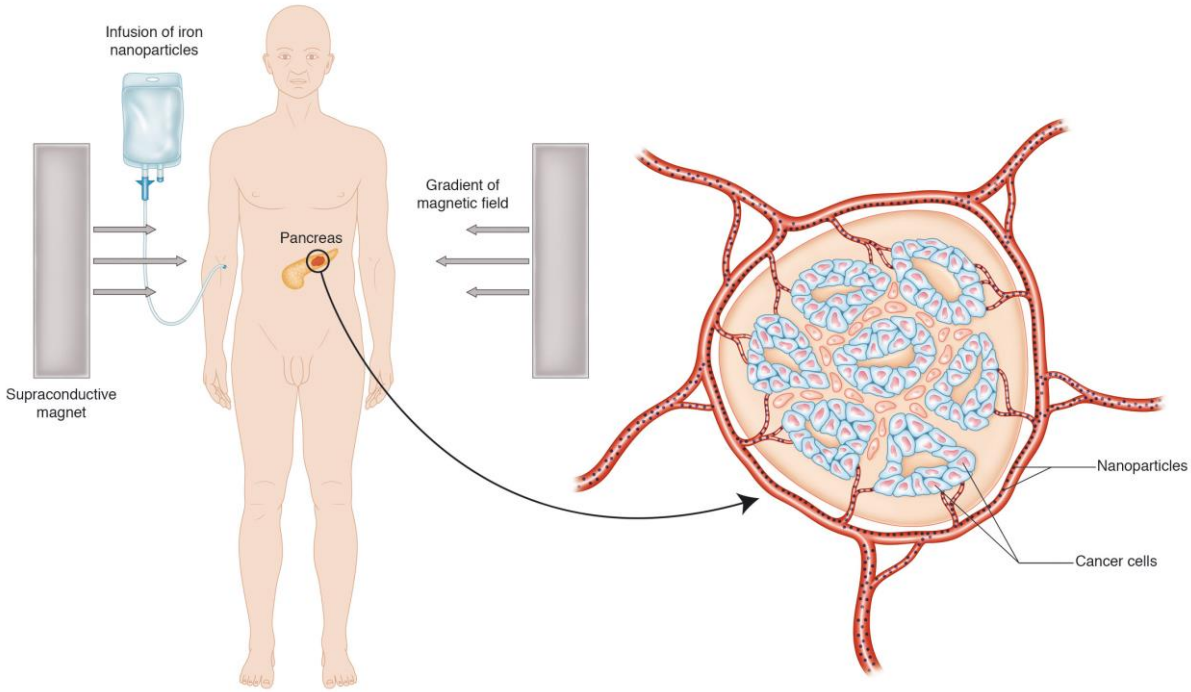


Figure 10

Diagram of a future treatment of a deep tumor

Make available, in the short term, a new treatment for pancreatic cancer; A decisive step for the In Vivo approach

The project of Proof of Efficacy will follow the Proof of Concept commented on page 19-21. The first step is to construct an animal demonstrator and test it on human pancreatic cancer grafted into the pancreas of mice (orthotopic graft) and show the action of a stress field in this case of deep graft, and no longer subcutaneous; this will be the "Proof of Efficacy". Then an industrial partner mastering the technology of superconductive magnets will be needed to move to the scale of the patient.

Recall that it is not a molecule, a drug product but the combination of two medical devices and the use of the laws of physics. So, there is only a few years of development before market entry; far less than the seven to eight years required for biological products.

Moreover, this process is based on the laws of physics: the results obtained and future can be modeled and are therefore predictive, further reducing the hazard associated with biology.

Moreover, the toxicity (a major cause of stopping the development of the molecules) is known here and a priori minimal. Indeed, magnetic field gradients are used in patients daily without side effects and injectable iron nanoparticles have been used as contrast agents, including vectorized particles for neoangiogenesis endothelial cells (PO 4 000, Chematech, Dijon, France; for animals).

Beyond Pancreatic Cancer

This type of treatment could be applied to all so-called locally advanced cancers and with no suitable therapeutic solution to date, such as brain cancer (glioblastoma) or primary liver cancer.

A priori any tumor located in the gradient can be treated. If the construction of an apparatus for including metastases in the volume treated is not trivial on the physical and mathematical point of view, it cannot be excluded from the outset.

What we learned from Mechanobiology in the Past

A well-known example: in order to repair a failing coronary artery it is commonplace to take a piece of vein from the patient and to graft it in the place of the artery (a ‘by-pass’ in fact). Under a much greater and pulsatile pressure, this piece of vein quickly acquires the structure of an artery: mechanical stress has caused it to pass from a vein organ to an artery organ.

Similar examples exist with bone and cartilage tissues.

Towards a Resonance Frequency?

The constancy of Young's modulus difference between cancer cells and normal cells independent of the original tissue type of cancer leads to the question of the therapeutic use of ultrasounds in the very high frequency range. Cellular models based on 'springs' and 'pots', or more sophisticated models, show the theoretical possibility of discrimination with respect to noise and between normal and cancerous. But to go further it will have to think resonance on the whole network support of the mechanotransduction and no doubt come out of the logic of destruction as therapeutic.

Towards a Paradigm Considering Mechanical Signals

The theoretical basis of carcinogenesis from cumulative mutations is solid and constant. But it is insufficient. The first flaw comes from the 'putting under the carpet' of everything that went against and that the first successes of genetics and molecular biology made marginal.

The second flaw is the reductionist approach confined in the biological/molecular domain.

Another disappointment comes from the great stagnation of the therapeutic application.

A Revisit of Carcinogenesis

The very first anomaly is the loss of parallelism of the mitotic spindle compared to the ECM.

For physicists, this transition from the normal to the cancerous is a ‘rough growth of a quasi-crystal’. It is difficult to reconcile these two conceptual models.

From the Surface of the Cell

If we summarize what the PO has told us:

Cells use transmembrane receptors, such as integrins, to mechanically couple their CSK to the ECM. Cellular CSKs are themselves linked together by other transmembrane receptors, such as cadherins, which interconnect cells of cancerous tissue. The assembly thus forms a network of transmission of the mechanical signals that extends through all the cancer organ.

It should be noted that transmembrane receptors are sensitive to forces or pressures but have few connections with the CSK that have purely local effects such as opening an ion channel, initiating signaling to the nucleus and thus transmitting only biological signals.

The CSK plays on its components, actin filaments, microtubules and intermediate filaments to control a change in stiffness - or vice versa - of the ECM.

The control will go through a rapid response from the CSK. Pre-stressed, this cellular and transcellular network channels mechanical signals: this allows the transmission of information not only to the nucleus and its (epi)genetic machinery, but also to the intracellular structures physically linked to the CSK such as the mitochondria.

This sort of pipeline avoids energy and signal losses and concentrates/channels the signal towards its biological targets: energy production by mitochondria and synthesis of proteins by the nucleus. The whole of the non-liquid structures of the cancerous or non-cancerous tissue, in fact soft matter, represents about 20% of the weight of the tissue but is the exclusive support for the transmission of mechanical signals. It adds cell and nuclear membranes, mitochondria, cytoplasmic CSK, nuclear CSK, the fibrillar network of the ECM.

The CSK pre-tension allows it to react quickly and in a finely modulable way to a force transmitted to it, and to modulate it according to its direction (pull, push, shear).

This prestressing is linked to the equilibrium between the three components of the CSK which are outside the scope of a study on the PO but have been the subject of an important literature. The prestressing makes it possible to better manage the efficiency of the maintenance of the cellular, tissue and organic architecture.

We shall not deal here with the emission of biological signals concomitant with the arrival of a mechanical signal: indeed, there is not only a biochemistry in the liquid phase but there is also a no less important solid-phase biochemistry located at unsoluble structures such as the CSK. For example, the activation of Rho, a small GTPase, is thought to induce contraction of CSK by genetic-biochemical pathways when stimulated by a force applied to an integrin. This contraction is then accompanied by a supra-molecular assembly with construction of focal points of adhesion that will strengthen intercellular physical communication.

Of course, the system is bi-directional and the fibrillar structure of the ECM will be modified in response to a transmission of mechanical signals coming from the cancerous tissue, resulting in a change in its mechanical properties. Thus, an increase in intracellular CSK tension will increase the tension of the ECM restoring the homeostasis of the organ.

For the panorama to be complete, mention must be made of the presence in large quantities of rigid filamentous structures which triangulate the cell membrane, anchored in the membrane bilipid layer. Similar organisms are found around the nucleus and in the mitotic apparatus during meiosis.

The main lines are drawn but the approach has been up to now more qualitative than quantitative.

The Stress Field and its Measurement In Vivo

The tissues of the organs are permanently immersed in a stress field. At any point of the cancer organ there are therefore force vectors whose direction and intensity can vary at any time. But we have seen that these forces are channeled into space by semi-solid structures and that these structures are prestressed, ensuring by this isometric tension the architectural stability of cells and tissues.

In vivo force measurement techniques are multiple with technique-dependent results and there is a lack of a unicist method.

Fractality of Cancer

All structures of the cancer organ are fractal on a micro and mesoscopic scale. Except the tumor itself which is spherical by default.

The surface of cancer cells (and this appears in vitro at a very specific time: when the 'transformed' cell becomes cancerous) is fractal as well as the growth border of the tumor. Tumor angiogenesis is fractal as well as the nucleus and architecture of cancerous tissue.

This fractal dimension has been used for the diagnosis of cancer: automatic reading of radios, biopsy samples, etc. with a very strong correlation between the fractality coefficient and the aggressiveness of the cancer.

The first application seems to have been the difference between the microcalcifications of benign tumors and malignant breast tumors on mammograms: those accompanying cancers have a fractal distribution in space.

These findings of the links between change of form and evolution of the phenotype do not exhaust a subject: to speak of fractals in biology immediately evokes the improvement of energy performance related to a fractal geometry. Thus, the fractal geometry of the bronchial tree optimizes the energy expenditure of respiration.

Cancer metabolism

Cancerogenesis and its progressive change of architecture modify many enzymatic functions including the metabolism that supplies their energy to the cells.

In an experiment published in 2011 F D'Anselmi et al have shown an original aspect of this 'Warbürg' phenomenon but in reverse: cancerous cells of human origin are reversed towards the normal. At the change of shape towards the sphere, order symmetry one, quantified by fractal analysis corresponds to a minimal energy state with decreased bending energy of the cell membrane. In parallel, the cancerous glycolytic phenotype collapses with decreased accumulation of lactate and fatty acids, then the synthesis of citrate decreases, etc. Until reaching the thermodynamic phenotype of the normal cell with a restored oxidative phosphorylation.

Entropic production is an index of metastatic potential, as well as entropic production per unit of time to fractality and cell growth rate; the study of mitochondria, which are truly cellular thermal power stations, is very fragmentary in this respect; Fluorine 18, a carrier for Positron Emission Tomography scan, has revolutionized cancer imaging, with fluoride signing the presence of a cancer-like metabolism: the heterogeneity of binding of labeled fluoride within a tumor measured by its fractality coefficient is an indicator of aggressiveness of cancer with a correlation with overall survival; changes in the fractality of chromatin facilitate its accessibility, heterogeneity of compaction, heterogeneity of the gene network and thus one can draw a regulation of transcription independent of the conventional regulation of the genetic code that can be used to test hypotheses of appearance of the Warbürg phenomenon.

One could multiply the examples of the use of cancer fractality but there is a lack of an overall analysis of the interaction fractality / metabolism in carcinogenesis in PO.

It can be seen that the PO is not limited to the application of mechanics. The thermodynamic approach must converge towards this new approach to oncology.

The abbreviations used

2D, 3D: In two dimensions: cell culture which spreads and adheres to the bottom of the culture dish. It should be noted that in humans there are rare epithelia in 2D at a single cell layer.
In three dimensions: cell culture that is organized in space to reconstitute a tissue. 'Organoids' are very similar

CSK: CytoSKeleton

AFM: Atomic Force Microscope

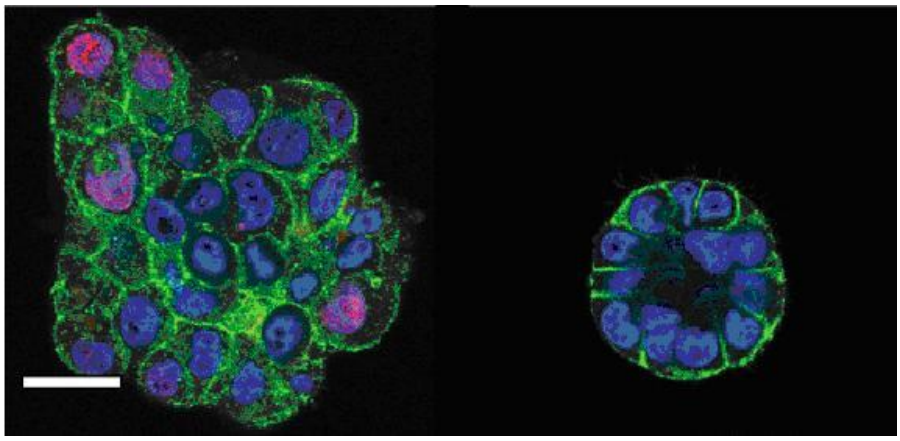
ECM: Extra Cellular Matrix

PO: Physical Oncology

Glossary

Acinius

The term acinus (plural acini or acinus) refers to a rounded epithelial cavity bordered by secretory cells which opens into the excretory canal of a gland.



Here we see a cancerous acinus on the right again normal to the left (publication of G Venugopalan, D Fletcher, 2012, American Society for Cell Biology) after physical confinement.

Atomic force microscope

The atomic force microscope is a type of local probe microscope used to perform local mapping of the physical quantities characteristic of the probed object (strength, capacity, radiation intensity).

Biopsy

A biopsy is the sampling of a very small part of an organ or tissue to perform examinations, the first of which is microscopic examination after staining. Biopsy is the prerequisite for any cancer diagnosis (hence the adage: no biopsy, no cancer).

Carcinogenesis

This is the whole process that leads from normal tissue to a cancerous tumor. The stages of the cancerization of an epithelium: dysplasia with or without metaplasia, in situ, primary tumor, metastasis.

Cytoskeleton and its components

The cytoskeleton is a network of microfilaments (microtubules, intermediate filaments and filaments of actin) present inside a cell and which ensures its rigidity

and the maintenance of its mechanical properties. The cytoskeleton is constantly built up. It manages the different intracellular movements such as the displacement of chromosomes during cell division for example. Likewise, it manages the deformation of the cell membrane and defends it against mechanical aggressions.

Drosophila

Drosophila or vinegar fly is, because of its ease of breeding, the model species in genetic research and the preferred tool of embryologists.

Epigenetics

Epigenetics is the study of the molecular mechanisms that modulate the expression of the genetic heritage according to the context.

While genetics is the study of genes, epigenetics is concerned with a "layer" of complementary information (eg, methylation of genes) that defines how these genes will be used by a cell or ... not.

Epigenetics was first demonstrated by cell differentiation since all the cells of a multicellular organism have the same genetic heritage, but express it very differently according to the tissue to which they belong.

Epithelium

Epithelia are tissues made up of closely juxtaposed (or joined) cells, without the interposition of fibers or connective tissues.

The cells are associated with each other through intercellular junctions. The epithelia are not vascularized. The contribution of nutrients and the export of waste are made in relation to the underlying connective tissue, via a basal membrane (of thickness between 50 and 100 nanometers) on which rests all epithelium.

In this type of tissue, the cells are polarized (the two opposite ends are morphologically and biochemically different.) In a unicellular epithelium, the apical region of the cell is easily distinguished and the basal region is joined to the basal lamina.

Euclidean

Euclidean geometry is based on the postulates of Euclid. She manipulates objects that are familiar to us: circles, triangles, rectangles, etc.

The fractals are like the Russian dolls, which contain an identical figure on the lower scale. This conception implies this tautological definition: a fractal object is an object of which each element is also a fractal object (similar).

Fibrosis, Inflammation

Fibrosis and inflammation are two vague terms, with multiple causes and varied consequences. Persists intuition that chronic inflammation could be a trigger for carcinogenesis, but all this is too vague to be useful.

Fractal

The adjective "fractal", from which usage has imposed the substantive a fractal to designate a figure or an equation of fractal geometry, is a neologism created by Benoît Mandelbrot in 1974. Many natural phenomena - such as lines Ribs or the appearance of the romanesco cabbage - have approximate fractal shapes.

Mitotic spindle

During cell division, the two sets of chromosomes are attracted in the two future cells by a bundle of microtubules which will pull the chromosomes in two opposite directions and strictly parallel to…

HER2

About 20% of breast cancers are called HER2 positive; The cancer cells carry on their surface this overexpressed protein.

Having positive HER2 cancer was a poor prognostic factor but today with the onset of trastzumab which strongly inhibits the function of this receptor, it has become a predictor of good response to treatment.

Histology

To examine a tissue under a microscope it is necessary to cut it into thin strips, to fix it in the paraffin, to color it and then to examine it. This is the work of the histologist. The histologist who examines the biopsies of the patients is a pathologist.

In Vitro, Ex Vivo, In Vivo

In Vitro: 'In glass'; The first cell culture dishes were made of glass. They are now plastic.

Ex Vivo: 'Outside the living' but tissues removed from the patient can be entrusted to the pathologist who will do the histological analysis, or kept alive for specific examinations.

In Vivo: 'In the living'

Mechanotransducer

Cadherins, integrins, vinculins... these proteins cross the membranes between cells and connect them to the ECM. One of their role is to trigger intracellular biochemical reactions, but they are also the link of the mechanical transmission between the outside and the inside of the cell.

Integrins

Integrins are cell adhesion receptors, ie transmembrane proteins, one end of which interacts in general with proteins/cells of the extracellular matrix located outside the cell (some integrins may interact with transmembrane proteins of neighboring cells), the other end interacting with intracellular constituents, in particular signaling molecules controlling migration, survival, proliferation and differentiation.

Integrins play a very important role in cell migration, differentiation and survival.

Basal Membrane

The basal membrane is a special extracellular matrix found at the interface between an epithelium and a connective tissue (often called a chorion). Its permeability regulates the exchange of molecules, in particular nutrients, between the two tissues. It contains a lot of collagen.

Neovascularization

Formation of new functional blood vessels irrigating a tumor (or other tissue abnormalities, in particular ocular abnormalities).

Young's Modulus or Modulus of Elasticity (in Pa)

It connects the stress applied to a material and its deformation

ExtraCellular Matrix (ECM) or Stroma or Conjunctive Tissue

The ECM represents a very large part of the cellular tissue of the organism, essentially constituted by cells, in particular fibroblasts which make another major constituent, fibers mainly of collagen, which give it a great resistance.

It is present in all organs except the brain and connects the organs together within the connective tissue.

Until recently it was presented as a 'support fabric' with no precise role. It is now at the heart of concerns for its role as a signal transmitter to epithelial tissue.

Metaplasia, dysplasia

In pathological anatomy, or histology - the two terms will be used here indifferently - metaplasia is the transformation of a differentiated cellular tissue into another differentiated cellular tissue. It is an adaptive and reversible phenomenon that occurs most often in response to repeated and prolonged tissue aggression (inflammation, mechanical or chemical irritation, infection). It accompanies many cancers.

We speak of dysplasia before an acquired alteration of the architecture and the function of a rapidly renewing cellular tissue (epithelium of coating, etc.). Dysplasia has a global tendency to progress to cancer, making it the sometimes abusive synonym for pre-cancerous conditions.

Micropatterning

In biology, micropatterning is the adaptation to the cell scale of an application to the cell of a geometric control of its adhesion to a culture medium and the control of the rigidity of its support.

Oncogenic

These are genes that control the synthesis of oncoproteins, proteins stimulating cell division or inhibiting programmed cell death (apoptosis), which triggers a so-called disordered (cancerous) proliferation of cells. The term oncogene may also refer to viruses which cause the appearance of cancers.

Organoid

In cell culture, an organoid is a three-dimensional multicellular structure that reproduces in vitro the microanatomy of an organ; It is therefore a model of the

organ (or a mini-organ). An organoid is generally obtained from one or more tissue precursor cells, embryonic stem cells or induced pluripotent stem cells, which can self-organize in three dimensions, in particular by virtue of their self-renewal and differentiation.

Techniques for the production and production of organoids from different tissues have developed rapidly since the years 2010.

Pascal (Pa)

The pascal, of symbol Pa, is the unit of pressure or constraint of the International System of Units (SI) in Newton per square meter.

Polarity

Cell polarity is defined in relation to the surface of the epithelium, the basement membrane and adjacent cells.

The apical pole is the cellular end towards the external environment.

The basal pole is the cellular end towards the inner medium.

The lateral faces are the ends towards the adjacent cells.

Cellular polarity is also defined by a characteristic distribution of certain organelles, certain enzymes and certain membrane receptors at the apical pole, at the basal pole or at the lateral faces.

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