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Review

Extracellular matrix, biotensegrity and tumor microenvironment. An update and overview

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Summary. The extracellular matrix (ECM) constitutes a three-dimensional network that surrounds all cells, organs and tissues in the body. It forms a biophysical filter for protection, nutrition and cell innervation, as well as the medium for facilitating immune response, angiogenesis, fibrosis and tissue regeneration. It is the mechanism by which mechanical forces are transmitted to the basement membrane which, through the integrins, supports the tensegrity system and activates the epigenetic mechanisms of the cell. A review and update on current knowledge on this topic reveals how disturbance of the ECM leads to a loss of efficient filtering, nutrition, elimination, and cell denervation functions, in addition to loss of regeneration capacity and disorders in mechanotransduction. Furthermore, such disturbance results in a loss of substrate, and with it the ability to provide a proper immune response against tumor, toxic and infectious agents. Reciprocal communication between ECM stromal and parenchymatous cells directs gene expression. The oncogenic capacity of the stroma derives from the associated cells as well as from the tumor cells, the angiogenic microenvironment and from an alteration in *tensegrity*; all of which are dependent on the ECM. It has been shown that the malignant phenotype is reversible by correction of the altered cues of the ECM.

Key words: Extracellular matrix, Pischinger basic system, *Tensegrity*, Desmoplasia, Cancer

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Introduction

Body tissues communicate within themselves at a higher speed to that provided by the nervous system. At histological level, these support and communication functions reside in the extracellular matrix (ECM), which is also responsible for the supply of oxygen and nutrients, as well as the elimination of CO_2, toxins and other waste materials. The toxins accumulated in the ECM are drained though the lymphatic system. When such drainage is insufficient, a response is produced, resulting in inflammation, acidosis and pain. The ECM is innervated through the vegetative nerve fibers; the correct function of which determines the predisposition of the matrix to respond with an inflammatory process to any nonphysiologic stimulus. Moreover, the ECM acts as a reservoir for numerous molecules, including growth factors, cytokines and proteases (Alvaro et al., 2009b, 2010).

The ECM accounts for 20% of our whole body weight and as such is the largest organ in our system. It is composed fundamentally of collagen, and constitutes the main structural support element in multicellular animals, performing a central role in tissue organization and orientation, as well as in cell adhesion, migration, differentiation, proliferation and apoptosis (Huxley-Jones et al., 2007, 2009). The ECM consists of a complex mixture of proteins, proteoglycans (PGs) and glycoproteins (GPs) that confer the structural properties of cells and tissues. Each cell presents its own receptor profile, creating a communication interface with the surrounding microenvironment. The ECM acts through these receptors to influence cell growth, death, adhesion, invasion, gene expression and differentiation processes. All these cellular events are present in the physiologic processes of embryonic development, tissue morphogenesis or angiogenesis. When the correct

information is lost, these events result in pathologic, autoimmune, inflammatory, degenerative and neoplastic processes as well.

Since Virchow first propounded the model of cellular pathology, it has been considered that the minimum unit of life in the organism is the cell. Nevertheless, a single cell cannot survive alone in isolation from its environment. In recent years, Alfred Pischinger has suggested the names 'the third system', 'the basic system', or 'the basal regulation system' to denote the entire structure surrounding the cell, defining it as a homeostatic system, and proposing the capillary-ECM-cell triad as the minimum unit of life in vertebrates (Fig. 1) (Pischinger, 2006). Several scientists have laid the foundations for knowledge of the ECM and have each contributed with essential features to the concept of the third system (Noble, 2008; Saks et al., 2009). Modern investigation does not understand the ECM as an inert material or a passive support tissue, but as a dynamic living component, possessing multiple functions, "a living matrix" (Oschman, 2009).

Study techniques and ECM histology

The techniques used for the morphologic study of the ECM, such as immunohistochemistry, immunofluorescence or electronic microscopy, require the fixation, processing and cutting of tissue, thus limiting the study of these structures and making dynamic observation *in vivo* impossible. The fine structure of the ECM is poorly preserved by histologic fixatives, usually appearing as a granular material between the cells and fibers. When using frozen tissue, the ECM is stained metachromatically with Periodic Acid Schiff's reaction (PAS).

Other techniques, such as *in vivo* confocal microscopy or multi-photon microscopy (MPM), facilitate the dynamic study of the ECM. MPM is able to utilize the properties of the collagen fibers of the matrix to generate a harmonic signal known as second harmonic generation or frequency doubling, allowing these fibers to be observed without the need to process the tissue (Friedl et al., 2007; Schenke-Layland et al., 2008). The elastic fibers of the ECM can also be studied with this method (Konig et al., 2005).

The ECM is the structural framework within which the support cells, the free nerve endings, the capillaries and the cells of the immune response system reside. It is constituted by fibers and by the ground substance, a viscous gel made of highly hydrated macromolecules

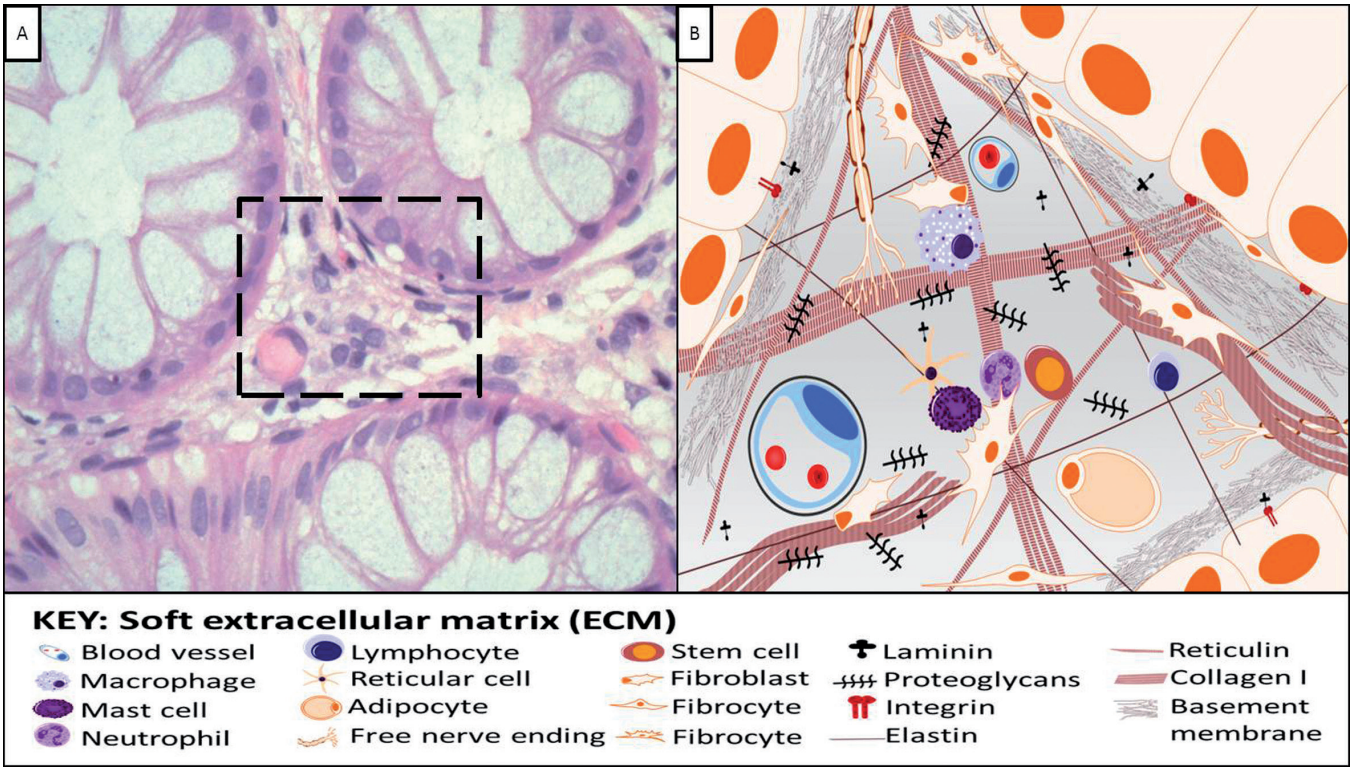

Fig. 1. A. Intestinal mucosae: Lieberkühn's glands and lamina propia. Hematoxylin and eosin stain. x 100. **B.** Schematic representation of the selected area in A. The capillary-ECM-cell triad is the minimum unit of life. It provides the structural support of *tensegrity*, a matrix through which the stimulus of innervation is transmitted and a capillary network to provide nutrition and to discard waste materials from the parenchymatous cells. It is also the framework for monitoring and action by immune system, as well as an energy flow network for high-speed local and distant communication.

(Geneser, 2000; Stevens and Lowe, 2006). The fibers confer tensile resistance and elasticity, and form a scaffold upon which the cells are arranged. The fine structure of PGs and GPs lies between a thick structure of collagen and elastin fibers, covered by interstitial or tissular fluid (Fig. 2).

The collagen system consists of different types of collagen fibers, each having their own structural and support function; reticular fibers, which are bundles of collagen fibrils smaller than 50 nm; collagens associated to fibrils, which bind the reticular fibers both together and to other ECM components; and anchorage collagens, which bind the type I collagen fibers to the basement membranes and laminas, among others.

Within the elastic system, thinner fibers branch out and bind together, forming a very irregular network.

PG monomers are formed by sulfated glycosaminoglycans (GAGs), previously known as mucopolysaccharides, linked to central proteins by covalent bonds. In form they resemble a laboratory brush, with a proteic central structure and the GAGs simulating the bristles. The GAGs are long, non-branched polymers of several disaccharides (up to 200 repeated saccharides), consisting of one uronic acid (almost always glucuronic acid) and one hexosamine (glucosamine or galactosamine).

The main GAGs are composed of hyaluronic acid, dermatan sulfate, keratan sulfate, chondroitin sulfate and heparan sulfate. The PG aggregates are structures formed by a molecule of hyaluronic acid to which the PG monomers bind transversely. The hydrophilic property of the ECM arises from the high overall presence of GAGs, consisting of very negatively charged polyanions linked by electrovalent bonds to a high number of cations, principally sodium, which, in turn, attract and retain a large quantity of water molecules. Any change in these electric charges would modify the hydrophilic properties of the ECM. The PG macromolecules occupy a large proportion of the ECM, forming domains or pores with a large, open helical configuration. The spatial organization and the negative charge of the PGs, together with an intrinsic turgor, facilitate the selective diffusion of the various molecules, creating a tridimensional network which acts as a biophysical filter opposing compression forces and counteracting deformation. The PG aggregates are found not only in the ECM, but also in the basement membrane (perlecan) or on cell surfaces (syndecan, betaglycan). PGs and GAGs synthesize rapidly, the fibroblasts are able to generate these structures in only 1 to 2 minutes, and their half-life is 2 to 120 days.

Glycoproteins contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains. Structural GP, which occur in connective tissue, help bind the fibers, cells and ground substance together. They may also help components of the tissue bind to inorganic substances, such as calcium in bone. Fibronectin, with binding sites for cells, collagen and GAGs, binds to the integrin cell receptors and links the cell to its extracellular environment. Laminin participates in this linking of cells and influences the filtration of molecules through the basement laminas. Nidogen, tenascin and vitronectin are others of the many existing structural GPs of the connective tissue.

The interstitial fluid is essential to maintaining the homeostasis between intracellular and extracellular areas. It is composed of a water vector containing mainly fatty acids, amino acids, sugars, coenzymes, chemical messengers such as cytokines, hormones, neurotransmitters, mineral salts and discard products.

The ECM has various structural patterns and a number of different biochemical compositions; one of these elements is the basement membrane, a highly complex and specialized structure. Epithelial cells adhere to the basement membrane through which they receive the controlling signals originating from the ECM. The basement membrane consists of a lamina lucida and a lamina densa, as well as GPs which vary depending on the tissue type: laminin, type IV collagen, nidogen and heparan sulfate-like PGs (Fig. 3). Among its functions we can highlight cell adhesion, regulation of proliferation and its role as a selective filter for molecular diffusion.

Biological and functional aspects of the third system

The living cell literally vibrates in permanent interaction with its surrounding environment. The formation of tissues and organs is based upon environment recognition, the distribution of cytoplasmic compounds, changes in shape, mobilization and directed displacement of cells; and the establishment of contacts and associations with other cells and / or extracellular materials which are scant in epithelial tissue and highly abundant in connective tissue.

The plasma membrane consists of a double lipid

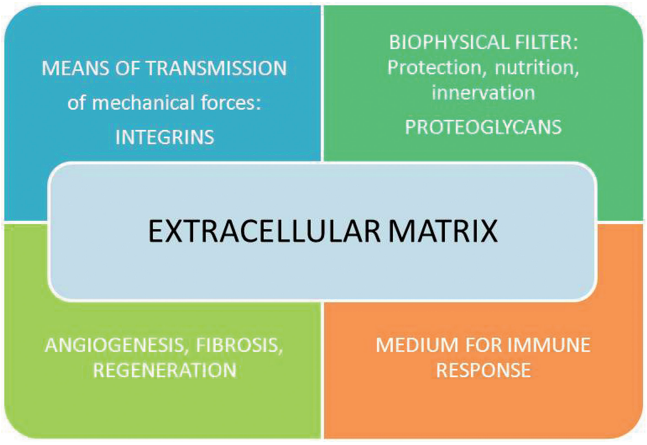

Fig. 2. Functional aspects of the third system.

layer and integrated proteins that traverse the membrane totally or partially. These components are able to make molecular movements such as turning, tilting from the outer to the inner surface and moving across the membrane. This explains cellular functions such as membrane flow, receptor function, cell recognition, surface enzyme activity, cell-cell and cell-substrate adhesion, cell motility in a liquid or over substrates, endocytosis and exocytosis, changes in cell shape, interactions and recruitment of ligands, and immune and histocompatibility phenomena.

The cytoskeleton is also a dynamic structure, made up of polymers comprising microfilaments, microtubules and intermediate filaments (Fig. 3). The cytoskeleton can be modified by extracellular changes in electric potential.

The main support cells are constituted by fibroblasts/fibrocytes, chondroblasts/chondrocytes, osteoblasts/osteocytes, myofibroblasts, adipocytes and gliocytes, which are essential for the synthesis of the extracellular fiber structures, as well as the PGs and the GPs. The quality of the biophysical filter formed by the PGs and the GAGs of the ECM depends on these support cells. Healthy support cells facilitate the rapid repair of the ECM after injury. The macrophages, neutrophils, mastocytes and other phagocytes remove the majority of unwanted substances, while the cytotoxic T lymphocytes and NK cells remove the poisoned or injured aberrant cells (Fig. 2).

The niches or biochemical and mechanical microenvironments are defined by coordinated competitive interactions between soluble factors, cells and the ECM. These niches are variable in both function and number, and can be created or altered under specific conditions, with a complex and dynamic regulation that influences stem cell transit, survival, self-renewal, proliferation and differentiation (Scadden, 2006; Discher et al., 2009).

Lymphoid cells are the main users of the communication highways of the ECM along which the lymphocytes flow easily at high speed (Korpos et al., 2009). When migrating they exhibit amoeboid movements which allow the cell body to align with the collagen fibers, using them as a physical scaffold to guide migration. This lymphocyte migratory movement is facilitated principally by Integrins which play a central role in this process (Lammermann et al., 2008; Sabeh et al., 2009).

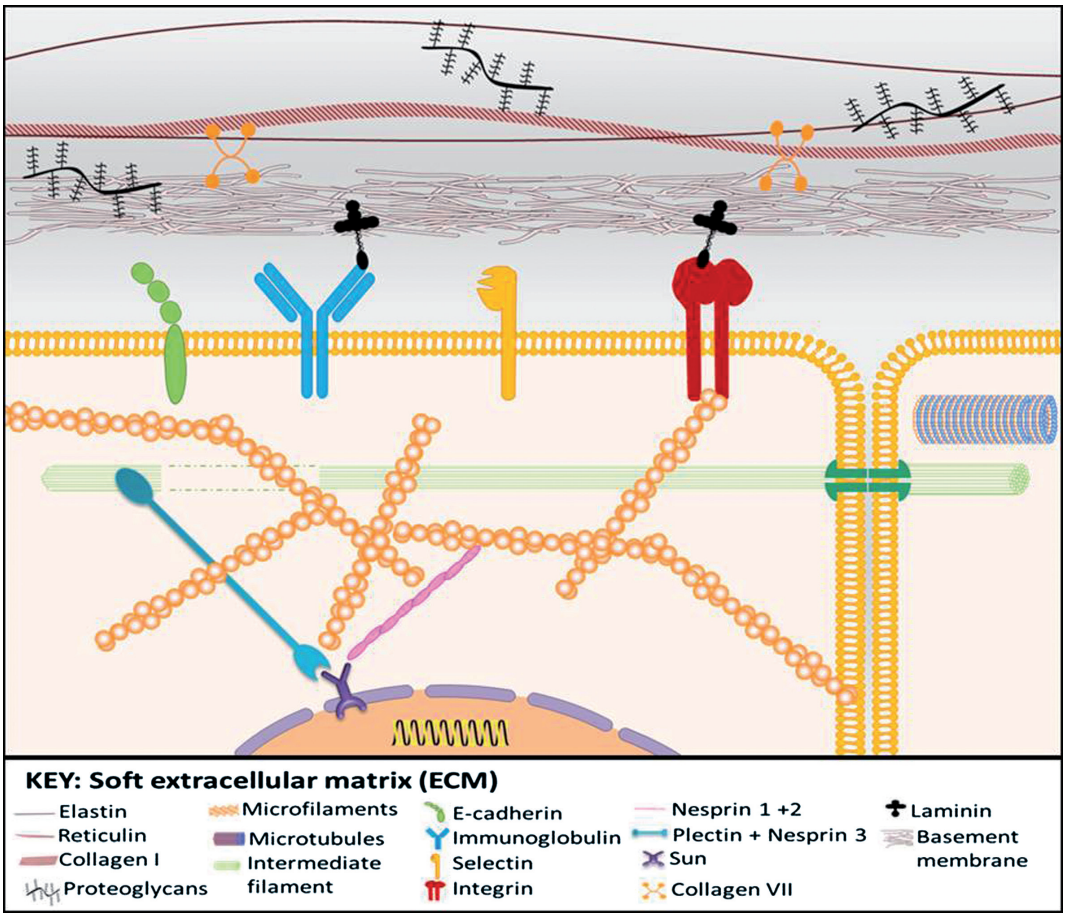

Fig. 3. The basement membrane. It provides structural support for epithelial cellularity. The cytoplasmic framework establishes connecting links between the nuclear matrix and the ECM through focal adhesions and integrins. The *tensegrity* and mechanotransduction forces are transmitted through the cytoskeleton; these forces give shape and movement to the cell, translate the biochemical stimuli and activate the epigenetic mechanisms of the cell.

CAMs (cell adhesion molecules) and Integrins

CAMs are a group of membrane GPs involved in biological processes entailing cell-cell or cell-matrix contact, such as proliferation, migration, differentiation and cell death. They recognize specific receptors, usually other CAM molecules located in other cells or in the cell matrix (Hynes, 1999). CAMs are grouped into families such as cadherins, immunoglobulins, selectins, integrins and ECM GPs. They can bind to molecules of a similar (homophilic interaction) or different nature (heterophilic interaction).

Cadherins are found on the surface of the majority of animal cells, and recognize other cadherins in adjacent cells. Immunoglobulin-type molecules establish homophilic bonds with immunoglobulins in the adjacent cells, whereas the selectins form heterophilic bonds, i.e., they bind to glucids present in the neighboring cell, and are important in the binding of leukocytes to the walls of the endothelium when they leave the bloodstream to enter the tissues.

Signals from the ECM molecules are transmitted to the surrounding cells through integrins which act as specialized mechanoreceptors. Integrins are able to translate and transmit a mechanical signal from the cell surface through a specific molecular pathway and convert it into intracellular biochemical changes, stimulate other receptors or induce gene expression. In addition, macromolecular complexes constitute adhesion points between the ECM and the cell, forming true mechanosensitive organelles or focal adhesions able to promote cell survival, the physiologic process of anoikis (apoptosis in response to an inappropriate relationship cell/ECM) and the proper replacement of tissue (Fig. 3) (Gilmore, 2005).

Integrins constitute the most important molecules involved in binding cells to the ECM. These molecules comprise a large family of transmembrane proteins that are expressed according to the type and physiologic needs of the tissues and cells, and possess an intracellular domain which is in contact with the cytoskeleton and an extracellular globular domain able

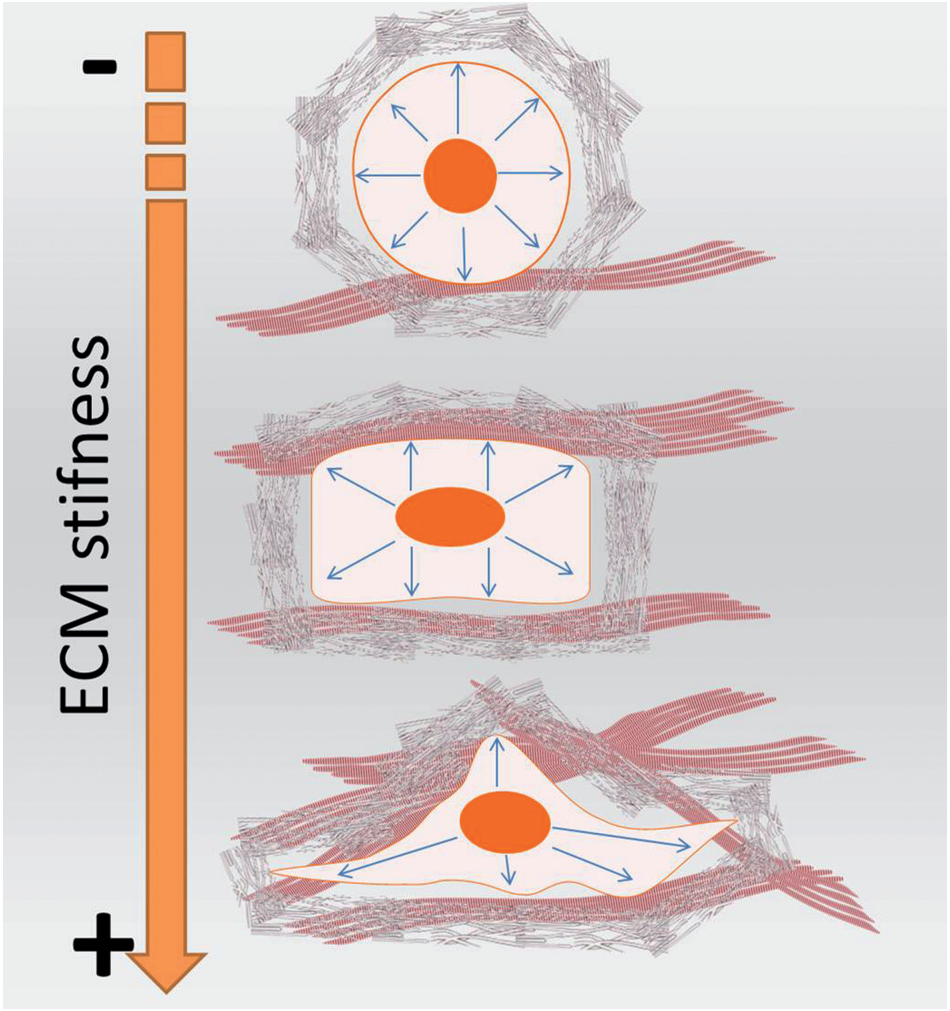

Fig. 4. *Biotensegrity*. The flattened or rounded morphology of the cellular elements and the three-dimensional structure of the tissue patterns, forming glands, alveoli, ducts and papillae, depend on the stiffness or flexibility of the basement membrane, as well as on its coordinated movement.

to bind to collagen, integrins and laminins (Akiyama, 1996).

The ECM provides a direct structural link between the nuclear matrix and the cell matrix (Getzenberg et al., 1991; Haque et al., 2006). The cytoskeleton (consisting of actin microfilaments, tubulin microtubules and intermediate filaments, specific for each cell type) is anchored to the nucleus through nesprins 1 and 2, which bind to actin filaments, whereas nesprin-3 can associate with intermediate filaments through plectin. Finally, the nuclear lamina forms a stable structure together with the nuclear structures, thus completing the mechano-transduction pathway between the ECM and the interior of the nucleus (Fig. 3) (Jaalouk and Lammerding, 2009).

Mechanotransduction and *biotensegrity*

Through the mechanotransduction process (Alenghat and Ingber, 2002), cells are able to convert mechanical changes into chemical or genetic changes. This process has been comprehensively studied in different cell types (Lin et al., 2009; Mammoto et al., 2009). In the body, bones constitute the basic compression support structure, forming part of a more complex framework in which all muscle, cartilage, ligament and tendons combine to form a tension structure. The fine balance between the forces that bind this framework together, through critical points such as joints, both holds the body together and enables it to move. Living organisms are holographic structures, systems within systems which repeat their properties at different levels. In fact the cell maintains its morphology and function through an integrated tension system, denominated *tensegrity*. Within the cell, microtubules constitute the compression structures, and the actin filaments, closely related to myosin filaments, the tension structures (Ingber, 2008). Mechanical tension generated by movement is transmitted by pressure to the ECM which in turn transfers the movement into the cell through the integrins. The cytoskeleton translates these forces into chemical signals and mechanical stimuli which are transferred into the nucleus through the intermediate filaments that connect with nesprins and Sun proteins. These in turn communicate with the nuclear lamina, and the nuclear lamina communicates with the DNA (Jaalouk and Lammerding, 2009). The nucleus itself also has its own *tensegrity* system, and activates proliferation, cell metabolism, differentiation or apoptosis through mechanical stimuli.

A system has evolved through which it is possible to activate different genetic cell programs by modifying the shape of a cell (Ingber, 1998). *Tensegrity* may offer a scientific explanation for the effect of massage and qigong (Manzaneque et al., 2004), or for the genetic modification of the cell as a response to relaxation

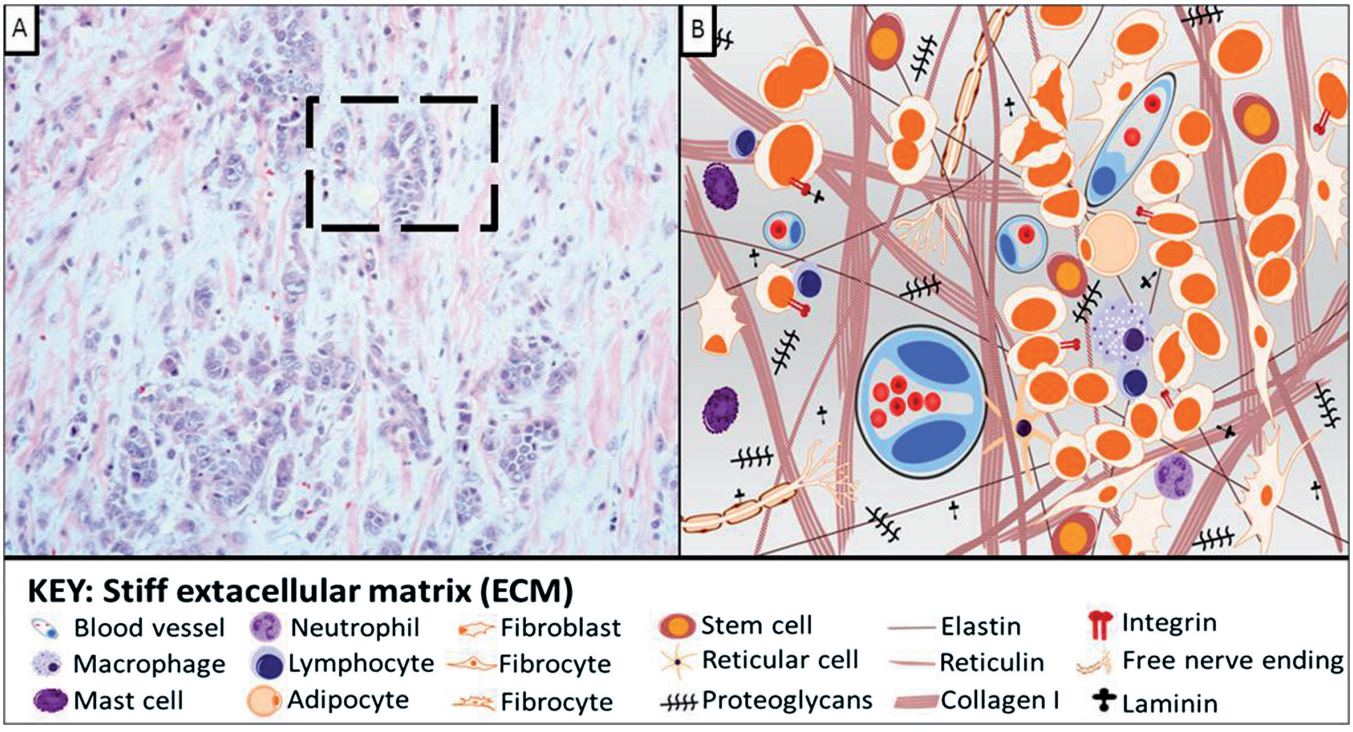

Fig. 5. A. Breast carcinoma. Hematoxylin and eosin stain. x 10. **B.** Schematic representation of the selected area in A. In this tumor, the oncogenic capacity of the stroma derives from the effect of its own cells, the lymphoid cells, and from the mechanical stimulation of *tensegrity* of the ECM. The loss of the tissue architecture causes a lesion to become a malignant tumor. ECM stiffness, the new-angiogenesis and desmoplasia cause an increased consistency that enables clinical detection of the tumor.

(Dusek et al., 2008). When a cell is flattened, the tension generated indicates that more cells are needed and the division process is activated. If the shape becomes rounded, the opposite process occurs and the apoptosis or anoikis program is activated. To establish equilibrium, the cell seeks the optimal conditions for differentiation and function according to the microenvironment in which it resides. All cells in the body are subject to this *tensegrity* mechanism based on the inner structure of the nuclear matrix and the cytoskeleton. Thus, every cell senses its environment and reacts to it according to its own needs (Fig. 4) (Ingber, 2003a,b, 2006; Discher et al., 2005).

Variations in the rigidity of the ECM allow it to produce a differentiated phenotype in the mesenchymal stem cells. A soft ECM with a pressure or 1kPa (kilopascal) would produce neurogenic differentiation; a stiff ECM of 10kPa would induce muscle differentiation (Engler et al., 2006). Such isometric tension is vital to life and explains why cells, organs and tissues take on a given shape, or why a hollow organ collapses when opened; this tension is found at the tissular, cellular, organic and molecular level, including the DNA structure itself (Getzenberg et al., 1991). A mechanical force applied to a living organ is transmitted down through a structural hierarchy that spans multiple size scales until becoming biochemical cellular stimulus through specific transduction molecules (Ingber, 2006).

The mechanotransduction process converts mechanical stimuli into chemical signals and allows the cell to adapt to its microenvironment (Jaalouk and Lammerding, 2009). This mechanism includes steps that pass from the ECM to the cytoplasmic membrane, the cytoskeleton and the nuclear membrane, and therefore involves an enormous amount of proteins and molecules. These elements have therefore been classified into three groups, comprising those of the extracellular microenvironment, those affecting the cell structure and organization, and finally, those related to cell signaling. All these proteins and molecules will eventually affect the nuclear chromatin at a genetic and epigenetic level (Wang et al., 2009).

Energy flow and physiopathology of the ECM

The electric and magnetic fields generated by tissues and organs possess an important biologic function. This function is altered in the pathologic processes of inflammation, degeneration or the appearance of new tissue within an organ. The electromagnetic activity of an organ is not only restricted to the boundaries of the capsule, but extends outwards involving the fields of neighboring organs, interrelating and communicating with adjacent and sometimes distant structures, similar to the electromagnetic wave sent by the heart throughout the body via the bloodstream. The essential unit of the ECM, the matrisome, hosts the function of maintaining the osmotic, ionic, electromagnetic and protonic homeostasis, both at local and systemic level.

Biological electricity is an ionic phenomenon linked to the polarity of the cell membrane, it occurs in nerve transmission, muscle contractility and in all living cells. These potential differences are easily measurable, although electrons and protons produce much smaller flows. Thus we have a system based on interaction of energy, which, together with the chemical system constitutes a language essential to the integrity of the body; an integrated network that links genes and nuclear matrix to the ECM and to its collagen fibers through integrins and focal adhesions. Knowledge of this structure provides histology with a clue to its function, allowing the visualization within tissues of the circuits of energy that support life and its relationships, integrated through the *tensegrity* and mechanotransduction mechanisms. Thus, the nuclear matrix, the cytoplasmic matrix and the ECM form an interconnected network linking all molecules in the body (Fig. 5) (Wang et al., 2009).

Energy pathways and information circuits are routed principally in the ECM, whose composition and structure not only provides a supportive element, but also precise circuits for mechanical, ionic, electrical, protonic and chemical transmission. The electric fields produced by movement provide the information that directs cell activity and the pattern of tissue remodeling (Bassett, 1993). The ECM is able to provide the means for high speed communication thanks to its hydrophilicity, which depends on the intense negative charges of its GAGs components. All diseases are accompanied by changes in ions and water content, and in pH levels of the extracellular fluids, thus affecting the cell membranes and their electric micropotentials (Goller et al., 1986; Kim et al., 2007).

ECM components are semiconductors able to transfer the electricity of the electrons and the proticity of the protons, which is another function of the ECM studied by quantum chemistry (Alvaro et al., 2009a). The ECM is a pool of negative charges able to donate or absorb electrons, used in the neutralization of free radicals released by oxidative processes, as in the case of inflammation.

The ECM determines tissue specificity and takes an active part in the development and maturation of the central nervous system, its remodeling, axonal guidance, regeneration after injury and the capacity for neural plasticity (Zimmermann and Dours-Zimmermann, 2008) (Huang et al., 2009). Furthermore, it can act as a stimulus for cell survival, as well as affecting its viability. The molecules of the ECM are related to the processes of cell life and death through the balance between metalloproteinases (MMP) and its inhibitors (MMPI), and can play an important role in tumor progression, inducing or suppressing apoptosis (Marastoni et al., 2008). The balance between MMP and MMPI affect different physiologic and pathologic processes, including embryonic implantation,

angiogenesis and carcinogenesis (Huang et al., 2009), thus constituting new potential therapeutic targets in inflammatory (Korpos et al., 2009) and tumor (Denys et al., 2009) processes. In prostate cancer the metastatic process is associated with changes in the ECM (Stewart et al., 2004); and in melanoma the degradation of the basement membrane and remodeling of the ECM are produced by MMP and its inhibitors (Hofmann et al., 2005).

The evidence available suggests that one of the mechanisms by which a lesion is converted into a malignant tumor is through the loss of tissue architecture (Fig. 6). The parenchymatous cells are anchored three-dimensionally through interactions with the ECM. This contact is essential for cell survival, to the point that anoikis occurs when a cell becomes detached from the network and is left with no locational reference (Gilmore, 2005); only metastatic tumor cells escape this law. The majority of malignant tumors show an increase in consistency due to the stiffness of their ECM. Mechanical changes to the matrix activate integrins, which not only promote cell proliferation through the Ras and Erk pathways, but also affect cell contractility through the Rho pathway (Huang and Ingber, 2005) whereby the greater the rigidity, the greater the stimulation of the integrins, which in turn increases stiffness, a fact that is reflected in the malignant phenotype of the epithelial tumors (Fig. 7).

The ECM, furnishing the tumor microenvironment

The concept of genetic determinism is currently under review given the evidence that tumors are heterogeneous cellular entities whose growth depends on the interaction between the genetically altered cells and the tumor microenvironment in which they develop (Ingber, 2008). This microenvironment consists of ECM, stroma cells and immune response, determining not only the morphology and the classification of the tumor, but also the clinical aggressiveness, prognosis and response to treatment (Alvaro et al., 2009a; Lejeune and Alvaro, 2009). A tumor could therefore be considered as a functional tissue, connected and dependent on the microenvironment which sends and receives signals to and from the tumor tissue itself. Thus, cancer may be a disease of neoplastic cell development, whose abnormal growth could be normalized through embryonic tissues or the ECM itself (Kenny and Bissell, 2003), changes in the ECM and in normal tissue architecture being as oncogenic as radiation or viruses (Ingber, 2008). The oncogenic capacity of the stroma derives from the effect of its own cells, the lymphoid cells, and from the mechanical stimulation of *tensegrity* of the ECM (Li et al., 2007).

Tumor stromal cells derive from bone marrow progenitors which are mobilized through the bloodstream until entering the tumor microenvironment (Roorda et al., 2009) where they differentiate following different cell pathways in endothelia, fibroblasts and histiocyte-macrophages, which control tumor cell growth, the metastatic potential of the tumor and also determine the response to treatment (Li et al., 2007). The collaboration of one of these cell types in particular, the macrophages, is essential in the processes of migration, invasion and tumor metastasis (Condeelis and Pollard, 2006); however it is the mesenchymal stem cells of the tumor stroma that drive the tumor cells to metastasize (Karnoub et al., 2007). Stem cells in the bone marrow are the precursors of metastasis to distant organs, and ensure the activation of an optimal microenvironment or niche to receive and host the tumor cells which arrive later (Kaplan et al., 2005; Hu and Polyak, 2008). Fibroblasts are the chief fabricators of ECM and are able to change their phenotype and function both in physiologic and pathologic contexts. In the tumor context we can find myofibroblasts, peritumoral fibroblasts, reactive stromal cells and carcinoma-associated fibroblasts (CAF) (Fig. 5) (Olumi et al., 1999) acting on tumor cells, as well as on the rest of the tumor microenvironment (Fig. 8) (Tlsty and Coussens, 2006).

The stromal cells and their products are able to produce cellular oncogenic transformation (Pupa et al., 2002), which acts through alteration in the homeostatic regulation of the tissue. Under normal conditions this regulation is responsible for maintaining architecture, adhesion, and programmed cell death or cell proliferation signals; so if the cells cease to receive this information from the ECM, firstly their function will be altered, followed by their structure and, finally, the oncogenic change will occur (Alvaro et al., 2009a). Remodeling of the tumor microenvironment will determine both the response to cytotoxic treatment and tumor prognosis.

The number of inflammation-derived mediators with the potential to promote cell proliferation, genomic instability and metastasis is virtually endless. Some examples are the release of cytokines and chemokines, the release of cytotoxic mediators including reactive

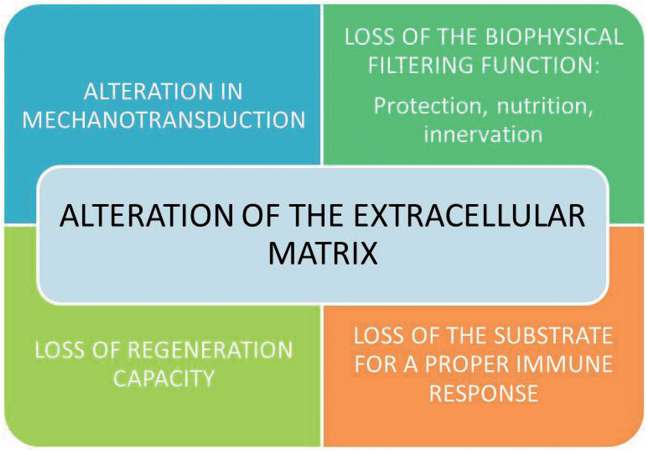

Fig. 6. Physiopathology of the ECM.

oxygen species, the secretion of proteases (especially MMP), soluble mediators of cell death and cell proliferation as TNF, interleukins, interferons and so on. These mediators are so abundant and have so many functions that the individual study of any one of these agents has so far produced little information. This circumstance calls for a reading at a higher level, that of the so-called hyperstructures, in an attempt to interrelate the data coming from the different fields of genomics, proteomics and all cellular physiology (Amar et al., 2002). The concept of hyperstructures considers the interaction of thousands of molecules, genes, ions, lipids etc. in carrying out a single tissue or cellular function. This interaction is influenced by extracellular regulatory signals proceeding from the microenvironment and the non-neoplastic cells of the tumor stroma, constituting the epigenetic framework for tumor progression (Huang and Ingber, 2006).

Transformation from a benign proliferative cell into a malignant cell can be produced by a peculiar phenotypic change, known as epithelial-mesenchymal transition (Guarino et al., 2007). This transformation involves breaking contact with sister cells and increasing motility, as the result of a change in the epithelial cytoskeleton, with its corresponding proprieties for a pseudomesenchymal phenotype, which enables migration, invasion and dissemination (Lee et al., 2006). While normal cells adhere to their environment through integrins, and their body has a proper consistency, tumor cells lose that consistency and *tensegrity*, becoming easily deformable elements (causing pleomorphism), with high elasticity (enhancing infiltration) and with an increased degree of mobility (enabling metastasis) (Suresh, 2007).

In lymphomas, the ECM regulators, especially MMP-9, influence angiogenic activity both at local and distant level (Negaard et al., 2009). Abnormal vasculature in the stroma, as well as fibrosis and increased interstitial pressure, confer the firmness of the tumor on palpation. Under homeostatic conditions, collagen fibrils are subject to a scant turnover. However, this turnover is accelerated during tissue remodeling and tumor development, as evidenced by the serum levels of its degradation products. A desmoplastic reaction is frequent in many solid tumors, such as breast, prostate, colon or lung, in which high levels of TGF-b and PDGF are found. These growth factors are produced by the mesenchymal cells of the tumor stroma and induce immunophenotypic changes. These changes are observable by studying actin-alpha, myosin, vimentin, desmin and the altered production of several ECM proteins, such as collagen, laminin, tenascin, MMP and MMPI, and several growth factors (Pupa et al., 2002).

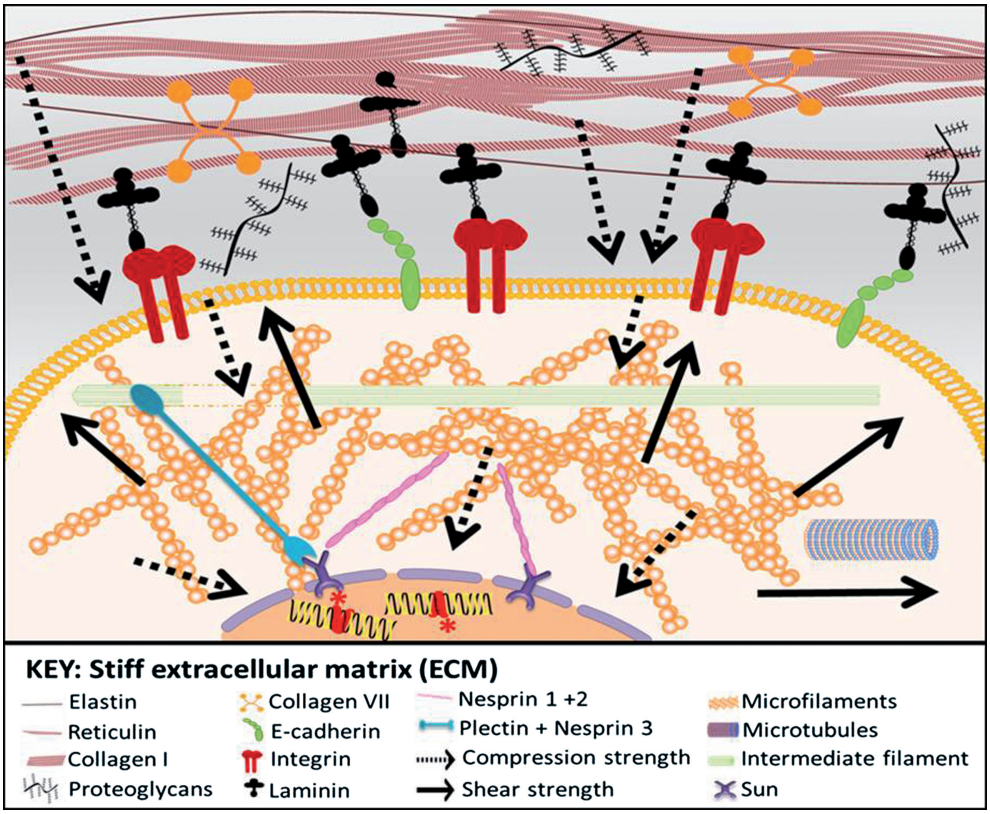

Fig. 7. The reciprocal communication between the stromal cells and the tissue parenchyma directs gene expression. E-cadherin is involved in the processes of tissue differentiation and morphogenesis and plays an important role in modulating the invasiveness of tumor cells in breast cancer and other epithelial tumors.

Studies of the ECM have revealed that the components of the tumor microenvironment are fundamental, not only for the regulation of tumor progression (Tlsty, 2001; Tlsty and Coussens, 2006), but are also essential even before the tumor appears. The stromal cells are able to produce the transformation of the adjacent cells through an alteration in the homeostatic regulation of the tissue, including the control of architecture, adhesion, cell death and proliferation (Pupa et al., 2002).

An overexpression of the ECM molecules themselves can increase tumor proliferation and confer resistance to chemotherapy, as in small cell lung carcinoma.

Molecular mechanics and therapeutic consequences of the regulation of the third system

Malignant transformation is accompanied by a progressive loss of local homeostasis and an alteration in tissue architecture. This culminates in tumor cell invasion and the production of distant metastasis. Throughout this entire process, a series of changes related to cell and tissue *tensegrity* are produced and result in a mechanical phenotypic change in both the tumor cell and its microenvironment (Figs. 7, 8). Micromechanical alterations of the ECM, together with remodeling of the stroma by the tumor cell, are linked to the phenomena of dysplasia, infiltration and metastasis (Kumar and Weaver, 2009). Knowledge of the molecular mechanisms by which a cell receives, processes and responds to mechanical stimuli is opening up new perspectives on the biology of cancer and its treatment. Alterations in the mechanical interaction between cells and their microenvironment contribute to cellular dysplasia. Conversely, the homeostatic regulation of the tensional force within the cell is able to reverse the malignant phenotype (Paszek et al., 2005; Johnson et al., 2007).

The emission of filopodia-like projections with a higher actin density, known as invadopodia, are used by the tumor cells to help digestion and invasion of the ECM, remodeling the existing matrix and establishing new pathways in the ECM which will be used for tumor invasion (Yamaguchi et al., 2005). During this action, the tumor cell will suffer significant cytoplasmic and nuclear deformations, with corresponding changes in organization of the cytoskeleton on traversing a rigid stroma. In turn, the expanding tumor compresses the adjacent ECM, with the consequent reduction of flow in

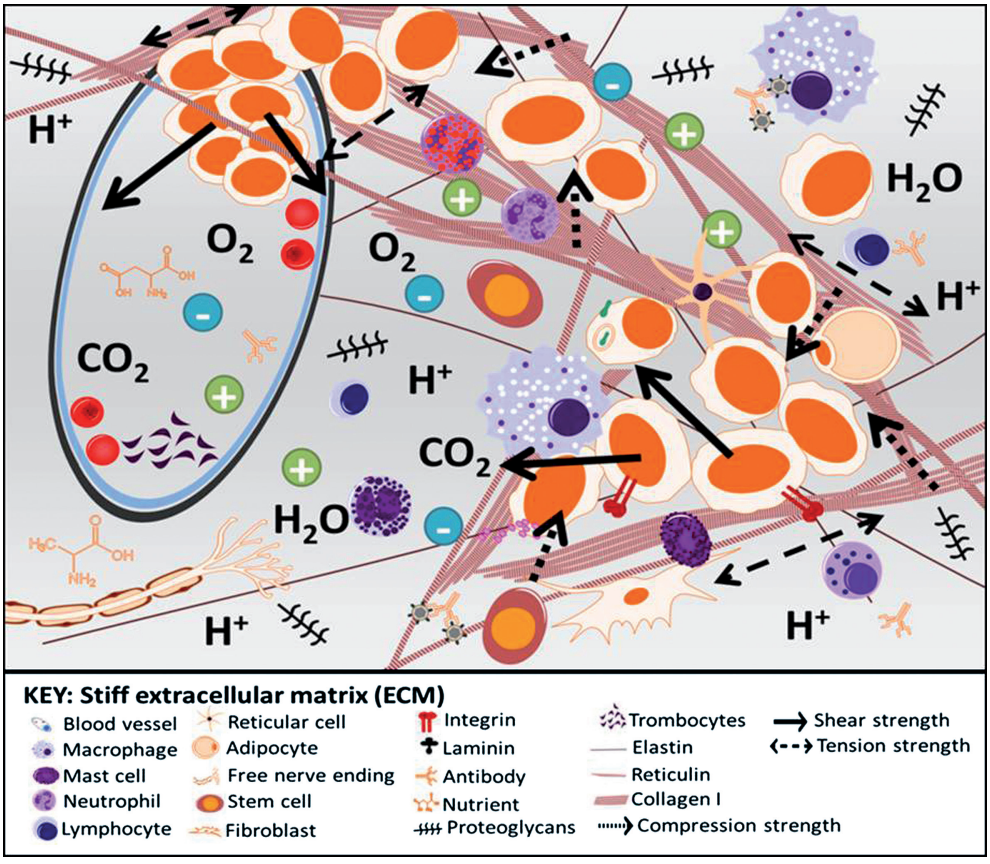

Fig. 8. Cancer cells, represented as the largest ovoid cells, often metastasize to different organs with very different microenvironmental and mechanical properties. The mechanics of remote tissues and the cancer cells could regulate cell dormancy, proliferation and differentiation in these organs.

the vascular and lymph vessels and of the interstitial space. Tumor growth is associated, first, to an increased matrix deposition and the adjacent ECM densification, and second, to a massive secretion of MMP and peritumoral matrix digestion.

Currently, two humanized monoclonal antibodies that act on the integrin interactions have been approved. Efalizumab interacts with the integrin lymphocyte function-associated antigen 1 (LFA-1) and is used in the treatment of patients with chronic plaque psoriasis, whereas natalizumab interacts with VLA-4 and is used in the treatment of multiple sclerosis.

With respect to the CAMs, a large number of investigations consider them to play a relevant role in pathology, and several projects to investigate new therapies with CAMs as the therapeutic target, usually by means of antibodies to modulate their action, are underway.

The perspective of the epithelial-mesenchymal transition in epithelial tumors has opened the door to a new line of treatment which considers the genetic and epigenetic mechanisms associated with resistance to chemotherapy (Sabbah et al., 2008). In breast cancer, treatment with doxorubicin results in an increase in fibuline-1, an ECM protein, and its binding proteins, fibronectin and laminin-1, which constitute a source of chemoresistance (Pupa et al., 2007). Monoclonal antibodies against fibulin-1 are able to reverse such chemoresistance, and the inhibition of MMP seems to have a therapeutic effect (Jodele et al., 2006).

Chemotherapeutic agents often base their cytotoxic action on the modification of cell membranes and the cytoskeleton. For example, doxorubicin produces peroxidation of the cell membranes, Taxol increases polymerization of tubulin, and vincristine impedes the addition of monomers to the microtubules, thus eliminating the possibility of mitosis (Suresh, 2007). In follicular lymphoma and diffuse large cell lymphoma, treatment with lenalidomide affects the immune synapses of intracellular T lymphocytes (Ramsay et al., 2009). Similarly, chemotherapeutic agents alter cell mechanics which increases the risk of vascular complications, as occurs in the treatment of leukemia. In leukemias, the treatment of tumor lymphocytes increases their rigidity due to the reorganization of the actin filaments, which in turn leads to a decrease in cell deformability with the consequent leukostasis, microcirculation obstruction and vascular complications (Lam et al., 2007).

Conclusion

Continued advances in cancer treatment require new and innovative approaches. The discovery that the malignant phenotype can be reversed through the correction of cues from the tumor microenvironment (Kenny and Bissell, 2003), paves the way for a new focus on experimentation and derived knowledge. Experimental evidence suggests that the cancerigenic effect of hormones on an epithelium occurs through an alteration in the stromal-epithelial interaction, and it has been shown that inducing changes in the ECM, *in vivo or in vitro*, is enough to influence the tumor phenotype. Nevertheless the opposite is also true, and some tumors may undergo regression by restoring an ECM which enables the proper interaction between the epithelium and the stroma (Ingber, 2008). The discovery that several cell behaviors are controlled by purely physical interactions has brought about a new era in which it will be possible to integrate current knowledge on the ECM and its function, together with the mechanisms of *biotensegrity*, mechanotransduction, energy flow, immune response and epithelium-mesenchymal transition. These advances will provide a truly comprehensive view of the neoplastic process, allowing a more specific and sensitive therapeutic management of the body and its diseases.

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