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Extracellular Matrix Remodeling and Development of Cancer

Koyeli Girigoswami[1] · Devender Saini[2] · Agnishwar Girigoswami[1]

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Abstract
The importance of stem cell growth and its fate is highly essential for the use of stem cells in therapy and regeneration. There are conflicting evidences regarding the actual role of stem cells when injected into a patient towards damage recovery and its lifespan inside the body. Tumor microenvironment differs from that of normal cells and may have a role in the growth of stem cells when associated with them. In cancer, the uncontrolled growth of cells remodels the extracellular matrix (ECM). The ECM alteration occurs as the mutated fibroblast cells release growth factors into the ECM which further alters the ECM directly or changes the epithelial cells and then alters the ECM. In this review we will discuss about the components and functions of ECM and how does it differ in cancer cells compared to normal cells. Abnormal dynamics of the ECM and its role in cancer progression will also be discussed.

Keywords Extracellular matrix · Stiffness of ECM · ECM remodeling · Cancer ECM · Cancer niche

Introduction

Tissue engineering is a field which resolves therapeutic problems such as organ failure, burn or organ transplants. The treatment modalities are executed with the help of organs engineered in the laboratory or by treating with the stem cells or with implantations such as biological scaffold (extracellular matrix, ECM) or artificial scaffolds (PCL, PLGA etc.). The implantation of scaffold work as a microenvironment for the cells and provides necessary signals and growth factors to the cells to retain functions. However, conflicts exist regarding the treatment by stem cells as there is no concluding study on the implanted cell survival after an interval of time. The damage recovery observed after the stem cell therapy also does not guarantee that the healing is due to the stem cells which were given to the patient. Because of these uncertainties stem cells therapy is under debate even after getting approved by the FDA. The study on the effect of cellular microenvironment becomes very important to come to any conclusion. The purpose of the present review was to explore the stem cell fate and its role in cancer, as it is well known that cancer microenvironment differs from that of normal cells, it may promote the growth of stem cells, which can be of future use for the stem cell therapy.

✉ Koyeli Girigoswami
koyelig@chettinadhealthcity.com

[1] Medical Bionanotechnology, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Chettinad Health City, Kelambakkam 603103, Tamilnadu, India

[2] Tissue Engineering and Regenerative Medicine, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Chettinad Health City, Kelambakkam 603103, Tamilnadu, India

Extracellular Matrix

Extracellular matrix (ECM) is defined as a compilation of molecules that are secreted extracellularly by cells to provide structural as well as biochemical support to the cells surrounding them [1, 2]. The ECM composition differs from one organ to another, but the basic functions of ECM include adhesion of cells, communication between cell-to-cell and also cellular differentiation [3]. The animal extracellular matrix contains the interstitial connective tissue matrix and the basement membrane [4]. The physical stress on ECM is managed by the compression and buffer like activity of the interstitial space which is composed of polysaccharide gels and fibrous protein [5, 6]. On the other hand, the epithelial cells rest on a sheet-like deposition known as basement membrane. Different kinds of tissue have their own specific ECM, like the bone tissue ECM are composed of collagen fibers and bone minerals; the loose connective tissue ECM contains reticular fibers and ground substances and the ECM of blood is the blood

plasma [7]. ECM with different characteristics and composition can play a role in many mechanisms like providing support to the cells, separate tissues and modulate communication within as well as among cells. ECM defines the behaviour of the cell; also, it sequesters extensive variety of cellular growth factors and becomes a niche for them [4]. Formation of these niches is due to the activity of activated proteases generated due to the changes in physiological conditions. This mechanism enables faster cellular function activation without *de novo* synthesis.

Mechanisms like fibrosis, growth, wound healing requires production of the ECM. As tumour and metastasis results in the alteration in the ECM using enzymes like MMPs, therefore aggressive tumour invasion and metastasis can be understood better by knowing the composition and structural properties of ECM [4].

The components of ECM are produced intracellularly by the cells and are secreted outside the cells through exocytosis for integration into the existing ECM (Fig. 1) [8]. Fibrous proteins and many glycosaminoglycans (GAGs) make network by interlocking with one another to form the components of the ECM. These components are discussed below:

Proteins

The different types of proteins present in the ECM is discussed below.

Collagen

Collagens are the principal structural proteins of the ECM and are categorized as fibrillar (collagens I- III, V and XI) and non fibrillar (Fig. 2). The fibrils of collagen provide high tensile strength to the ECM. Even, in humans, collagen is known to be the most abundant protein [9, 10] and also 90% of bone matrix protein content is collagen [11]. Procollagen is the precursor form of collagen, which is lysed by the action of proteases to allow extracellular assembly. Genetic effect in the genes which encodes this protein may result in disorders like Ehlers Danlos Syndrome, Osteogenesis Imperfect and Epidermolysis Bullosa [8].

On the basis of shape and structure collagen is of following types:

1 Fibrillar (Type I, II, III, V, XI)
2 Facit (Type IX, XII, XIV)
3 Short chain (Type VIII, X)
4 Basement membrane (Type IV)
5 Other (Type VI, VII, XIII).

Elastin

Elastin gives elasticity to the tissues, which allow them to stretch when it is necessary and can again come back to its original state. This property is useful in the lungs, skin, blood vessel, and ligamentum nuchae. These types of cells contain more number of elastins which are synthesized by smooth muscle and fibroblasts. Tropoelastins are the precursor form of elastins secreted in chaperone and this tropoelastin further produce progenitors when comes in contact with fibres of the matured elastins. Tropoelastins then chemically change into the elastin strand. Deficiency of elastin in the ECM causes disorders like cutis laxa and Williams syndrome [8].

Fibronectin

Fibronectins are the glycoproteins present in the ECM that connects the cells with the fibers of collagen and provides facilitation of the cell through the ECM (Fig. 2). Fibronectins attach to the collagen and integrin and remake the cytoskeleton to modulate the movement. Cells produce an

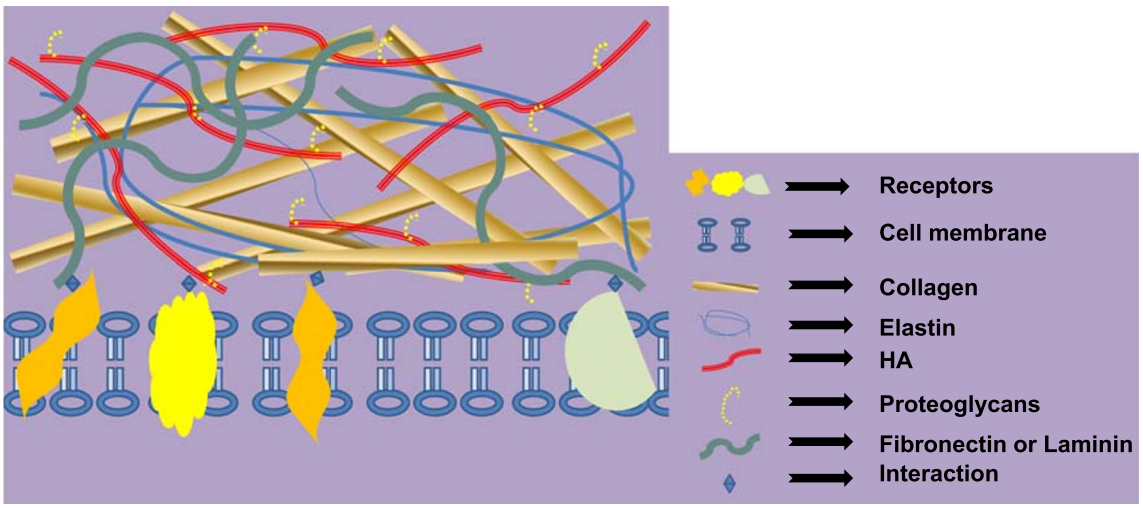

Fig. 1 The schematic diagram of extracellular matrix

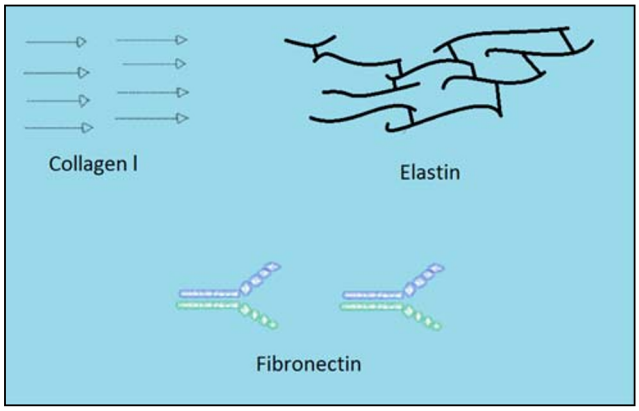

Fig. 2 Schematic representations of major protein components of ECM

inactive unfolded form of fibronectin. Unfolded fibronectin molecules make dimers by binding to integrins to retain their function. In blood clotting, fibronectin binds to platelets and modulate cell movement to the damaged area for wound healing [8].

Laminin

Laminins are the glycoproteins present in the basal laminae, and unlike collagen, laminins do not form fibers. They constitute networks of web-like structures which help in resisting the tensile forces in the laminae. Laminins connect to other ECM components like collagen and also helps in cell adhesion [8].

Thrombospondins

Thrombospondins (TSP) are the matricellular glycoproteins constituted of a family of five thrombospondins − 1–5 with two subgroups. These glycoproteins have antiangiogenic functions. TSP-1 can inhibit the proliferation as well as migration of endothelial cells after interaction with the CD36 expressed at cell surface. This interaction of TSP-1 with CD 36 leads to the expression of Fas ligand (FasL) which leads to the activation of caspases leading to apoptosis. Tumours which have TSP-1 overexpression lead to slower growth, lower angiogenesis and decreased metastasis rate [12].

Tenascins

Tenascins are the ECM glycoproteins which are abundant in the vertebrate embryo, wound healing sites, stroma of few tumours. Four members of Tenascin gene family is present namely: tenascin-C, tenascin-R, tenascin-X and tenascin-W. Tenascin-C is mostly studied and it exhibits anti adhesive properties which makes the cells to attain a round shape after addition to the medium [13]. The probable mechanism for this phenomenon may be due to the binding of Tenascin-C with ECM fibronectin and further blocking the interaction between the fibronectin with syndecans that help the cells to adhere to the matrix [14].

Nidogen

Nidogens are also known as the entacins which belong to the family of sulphated monomeric glycoproteins present in the basal lamina. Two types of nidogens are found in humans: nidogen-1 (NID1) and nidogen-2 (NID2) and are known to play important role in organogenesis during late embryonic development, especially for lung and cardiac development [15].

Proteoglycans

Proteoglycans are heavily glycosylated proteins, having a basic structure of a core protein (aggrecan, versican, perlican and decorin) attached covalently to one or more Glycosaminoglycans (GAGs) and are dispersed intermittently between the collagen strands. GAGSs are polymers of carbohydrate often found attached with ECM proteins to constitute proteoglycans. However, hyaluronic acid is noticed as an exception [7, 16].

The negative charge possessed by proteoglycans attracts the sodium ions which are positively charged and further attracts the water molecules through osmosis for maintaining moisture for the ECM and cells. Proteoglycans may play a role to help trapping and storing the growth factors within the ECM [7].

The various types of ECM proteoglycans (Fig. 3) are discussed as follows:

(a) Heparan sulfate

Heparan sulfate (HS) is a kind of linear polysaccharide found in every single tissue. It exists as a proteoglycan (PG)

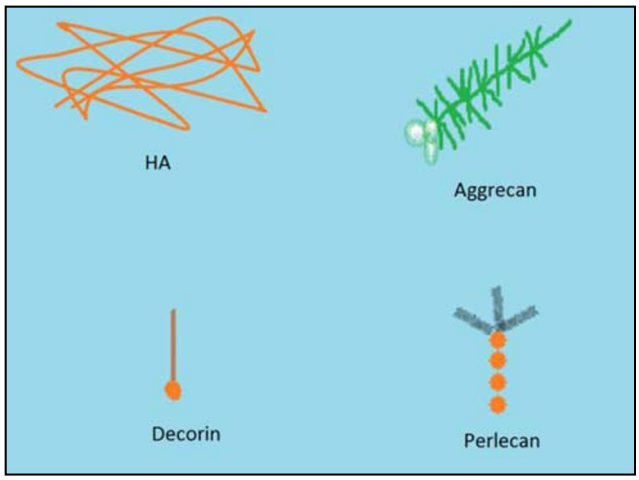

Fig. 3 Structural representations of some proteoglycans

in which a few HS chains are appended in closeness to cell surface or the proteins of ECM [17, 18]. HS being a member of GAGs has a close structural similarity with heparin. Heparin and HS both contain a variable sulphated repeating disaccharide unit. In HS the most common disaccharide is glucuronic acid (GlcA) linked to N-acetylglucosamine (GlcNAc) which contribute to a total of 50% of the disaccharides [7].

This form of HS binds to an assortment of protein ligands and are capable of regulating a wide assortment of biological functions such as development processes, blood coagulation, metastasis, angiogenesis etc. HS basically present in the ECM is known to attach with the proteins such as perlecan, agrin and collagen XVIII [19].

b Chondroitin sulfate

D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc) are the two monosaccharides combined to form linear chain polysaccharides of different length are known as Chondroitin Sulfate (CS) [20]. As a part of the proteoglycan, CS binds with proteins. CS plays a role in providing the tensile strength to tissue like cartilage, walls of aorta, ligaments and tendons. Neuroplasticity is also known to be influenced by CS [21].

c Keratan sulfate

Keratin sulphate (KS) is a sulphated glycosaminoglycan with linear polymer consisting of repeated disaccharide units like 3Galβ-1-4GlcNAcβ1. These can be modified with sulphate at carbon 6 (C6) of either the Gal or the GlcNAc monosaccharide. KS can contain different core proteins like lumican, mimecan, osteoadherin, keratocan, fibromodulin and aggrecan. The primary structure of KS has three regions namely, linkage region, repeat region and chain capping region. These are the sulfate components present in the cornea, bone and cartilage tissue. The amount of sulphate present in KS varies and unlike other GAGs uronic acid is not found in KS. In case of post trauma injury of brain, KS is synthesised in the central nervous system and there it contributes in the development and in the formation of glial scar. They are known as highly hydrated molecules which makes them capable as shock absorbers in between the joints [22, 23].

d Non-proteoglycan polysaccharide

The non proteoglycan polysaccharide present in the ECM is hyaluronic acid (HA). HA is also comprised of alternate residues of D-glucuronic acid and N-acetylglucosamine, but unlike other GAGs, it is not considered as a proteoglycan. HA present in the extracellular space helps the tissues to resist purse (press and squeeze at the same time). This is done by giving a counteracting turgor (bulging) force by absorbing huge amount of water. Load bearing joints like knee joints contains a large number of this component. HA is usually found to be present on the inner side of the cell membrane and during biosynthesis gets translocated outside the cell [21]. HA modulates cell behaviour in mechanisms such as inflammation, healing processes, embryonic development and tumour development [24].

Extracellular Vesicles

In 2016, one study found that within the ECM bio scaffolds, DNA, RNA, and Matrix-bound nanovesicles (MBVs) were present. MBVs consist of a variety of components such as proteins, lipids, fragments of DNA, and miRNAs. As ECM bio scaffolds controls regulation of mechanism in cells, similarly MBVs also can modulate the activation of macrophages and modify various cellular functions such as cell proliferation, migration and cell cycle. MBVs are presently accepted to be a basic and utilitarian key part of ECM bio scaffolds [25].

Physical Properties of ECM

Stiffness and Elasticity

The stiffness as well as elasticity of ECM differs from tissue to tissue. The elasticity of the ECM can fluctuate by several orders of magnitude. The concentration of collagen and elastin defines this property of ECM [26]. Cells can detect the mechanical characteristics of their surrounding by applying forces and subsequently assessing the backlash [27] which is essential to regulate contraction, migration, proliferation and differentitation [28–32]. Most of these effects are inhibited by the nonmuscle myosin II, which reveals that these phenomenons are related to the mechanical characteristics of ECM sensing [28, 29, 31].

Functions of ECM

Effect on Gene Expression

ECM with different mechanical properties influences cell behaviour as well as gene expression. It is not clarified that how this mechanism actually works, but it has been predicted that the contractile forces of adhesion complexes as well as the actin-myosin cytoskeleton, are propagated through the transcellular structure and are thought to play key roles in the molecular pathways [28].

Effect on Differentiation

Cellular differentiation can be controlled by the ECM elasticity, for example, mesenchymal stem cells (MSCs) show specific lineage and undergo specific phenotypic expressions with tissue-level elasticity of its ECM. MSCs plated over soft surface mimicking the brain surroundings gets converted into neuron-like cells with similar morphology, RNAi profiles, markers for cytoskeleton, and levels of transcription factor. On the other hand, MSCs when cultured over stiffer surfaces mimicking muscles and bones differentiate into myogenic, and osteogenic phenotypes [31].

Durotaxis

Durotaxis is known as the cell migration guided by stiffness and elasticity. Durotaxis was defined by researchers when they found that single cells started to migrate from low stiff surfaces to higher stiff surface [29] and focal adhesions (a huge protein complex acting as a primary contact site between ECM and cells) are thought to be the molecular mechanism behind such migration [33]. Proteins which are essential for durotaxis present in this complex are, integrins and signaling proteins such as FAK, talin etc. These proteins are responsible for the changes in shape of the cell and contractility of actomyosin [34]. The changes further control the directional migration by rearranging the cytoskeleton.

Cell adhesion

Numerous cells bind to different parts of the ECM. There are two mechanisms with which cells can adhere to the ECM. First one is by focal adhesion in which components of the ECM binds with the actin filaments present over the cell surface. Second is hemidesmosomes where the intermediate filaments connect the cells to the ECM. Cellular adhesion molecules (CAM) also known as integrins present over the cell surface, controls this cell-ECM adhesion. These cell-surface proteins (integrins) adhere cells to ECM components, for instance, fibronectin and laminins, and also to the integrin proteins on other cell's surface. The binding of fibronectins with ECM macromolecules facilitates the binding of these macromolecules with transmembrane integrins [5].

Remodeling of ECM

ECM components continuously interact with epithelial cells by acting as cell receptor ligands (like integrins) and transmit the signals for the regulation of survival, migration, adhesion, differentiation, proliferation, apoptosis. Growth factors, such as, fibroblast growth factor (FGF), epidermal growth factor (EGF) and other molecules of signalling pathways (transforming growth factor (TGFβ), amphiregulin) are sequestered and locally released by the ECM. The architecture of ECM as well as behaviour of cells, both gets regulated by the components released through ECM cleavage [35]. Cells constantly modulate the rebuilding and remodeling of the ECM by chemical modifications and synthesis, degradation and reassembly [36]. These mechanisms are very complex and are very precisely regulated to maintain the tissue homeostasis, especially, while responding to the injury. Uncontrolled remodeling of the ECM may result in pathological conditions and can generate diseases. For example: osteoarthritis is linked to the excessive degradation of ECM [37] and in case of cancer and fibrosis, abnormal deposition and stiffness is observed [38].

Cancer and ECM

To obtain detailed information about the behaviour of cells regarding its functions such as cell proliferation, differentiation, cell migration, cell morphology and growth, it is important to study the extracellular matrix (ECM) composition. Most ECM are composed of mainly collagens, elastins, and proteoglycans. Every component has a specific function, such as, collagens provide strength to the tissue, elastin and proteoglycans help cells to recover from the damage [39]. The biophysiochemical machanisms of the ECM, controls the fate of cells using its component proteins and physical properties [40]. The fate of cancer cells is also affected by the ECM and studies have shown that when cancer cell is implanted in blastula, it responses to the embryonic rules and behave similar to other cells in the tissue during development. The same study has also shown that in the regeneration process of mammary gland, the mouse testicular and neural stem/progenitor cells also resulted in the generation of mammary epithelial cells [41–43]. Normal generating tissue can control the cancer phenotype through its signals, which was concluded after an *in vivo* experiment in rat model where tumorigenic liver cells were implanted in a healthy rat liver resulting in the differentiation into normal liver cells [41–43].

ECM is pleiotropic in nature and affects the cell fate through different mechanisms. For example, matrix controls the stability and bioavailability of the growth factors, cytokines etc. which bind to it [35, 40]. Researchers have found that in a transgenic mice model, overexpression of ECM degrading enzyme MMP3 in the mammary stroma, developed mammary cancer after a time interval which proved the importance of ECM for the cell behaviour and function [44]. In tumour, the composition of tumour microenvironment (ECM) is by tumour cells and stromal cells in combined contribution [45]. In one study, different types of human breast cancer cells were injected in mice and observed. The results showed that some cells have enhanced metastatic potential and they

have spread to the other parts of the body while some showed less spreading rate. When proteomics was done to identify the proteins, which made the ECM of these tumours, it was found that each ECM has some common proteins. Some proteins were found only in the ECM of tumour cells with low metastatic potential and these proteins were absent in the ECM of tumour cells with high metastatic potential and vice versa. Study also concluded that within the tumour, stromal cells along with the tumour cells contributed to the composition of tumour ECM and the role of non cancer cells also differed in the different ECMs [45]. Some studies suggested that cancer can be controlled to produce normal cell types by changing the microenvironment or can be reverted into normal phenotype by altering the signalling of the matrix [46].

ECM can design the structure and organization of tissue and also helps in migration of cells, signals, growth factors, cytokines, etc. and regulates the interaction like cell-cell interaction, cell-ECM interaction, etc. In case of spinal cord or brain injury, inflammatory cells further damage the ECM by releasing ECM degrading enzymes matrix metalloproteinases known as MMPs, and due to the damage or alteration in the ECM, the migration, interaction, survival of the cells gets affected [47–50].

Composition of Normal and Tumorigenic ECM

Tumorigenic ECM differs from normal ECM in topography as well as physical strength, biochemical properties and other parameters. For example, collagen in tumour ECM deposits in a very high amount in the early cancer progression [51, 52] and plays main role in tissue stiffness which trigger the signalling in the non cancer cells and tumour epithelium [52, 53]. The collagen processing of ECM is catalysed by specific proteinases, for example, lysyl oxidases and lysyl hydroxylases, which catalyse the crosslinks in between intramolecules of collagen and elastin that further change the cell behaviour by modulating the elasticity and strength of ECM [52]. Any type of modification or increase in the crosslinked matrix makes the tumour tissues stiffer and increases the main signalling pathways activity which are responsible for cell growth, survival and migration [47, 54–56].

In the breast tumour ECM, collagen I is often highly linearized rather than being nonoriented fibrils. The linearized collagen I is aligned along the epithelium or can be projecting perpendicularly into the tissue [53, 57]. The consistent change in these architecture as well as the physical properties of the ECM, the expression of the enzymes related to ECM remodeling are often downregulated in human cancers. In many cancers, the cysteine cathepsins, heparanases, 6-O-sulfatases, urokinases, and MMPs were found to be overexpressed [58, 59]. Also, such diseased conditions like cancer result ed changes in the biomechanical properties of the ECM. For example, stroma of the tumour tissues is stiffer than that of normal stroma (for example, in case of breast cancer, 10 times of that in normal stroma) [53, 60]. This increase in the stiffness of the cancer tissue can be attributed to the over activity of lysyl oxidase (LOX), which makes cross linkage to the collagen fibers as well as other components of the ECM. In case of cancers like, breast cancer, head and neck cancer, expression of LOX were found upregulated [61, 62]. Inhibition of LOX in Neu breast cancer model showed to reduce tissue fibrosis and tumour incidence [53]. Altogether, these data suggested that downregulation of collagen cross liking and stiffness contribute in the cancer pathogenesis. However, LOX overexpression is not the only cause for cancer, remodeling of the ECM is also an important contributor [53].

Abnormal ECM Dynamics During Cancer Progression

Multicellular organisms have undergone evolutions in many redundant methods to prevent a cell which is highly integrated along with other cells inside a completely functional tissue, from converting to a cancer cell. Thus, to overcome this protective shield and proceed for cancer, cells have to acquire multiple oncogenic properties that can cause malignancy. The oncogenic properties include, the ability of grow, invade and survive [63, 64]. Cancer cells also lose their abilities like differentiation state and polarity, the integrity of tissue gets disrupted, and stromal cells get corrupted to enhance their own growth in primary tumour as well as at distant sites [65, 66].

An ECM which is abnormal in nature can promote several of the steps mentioned above. Integrin signalling can be up regulated by means of increase in the collagen deposition alone or in combination with stiffness of ECM. This further helps in enhancing the proliferation and cell survival [55, 67]. Overproduction of LOX results in increased cross-linking of the collagen and ECM stiffness, which further promotes focal adhesion assembly, pl3 kinase and ERK signalling thereby facilitating Neu-mediated oncogenic transformation [53]. However, various ECM components have pro or anti-apoptotic effects [68]. Therefore, apoptotic evasion of mutant cells is followed by the deregulation of the ECM remodeling.

Discussion

The promising role of stem cells in the treatment of many diseases mediated via endogenous cell repair promotion or through tissue regeneration by direct cell implants has been immensely studied in the recent times. To enhance the potential of stem cell technology it is important to understand the fundamental signals and mechanisms that can control their behavior. ECM plays a pivotal role in deciding the fate of the stem cells and the physical properties of the ECM highly contribute towards the stem cell fate. ECM is a fibrous

network in a gel-like material having multiple functions and provides biochemical as well as structural support to various tissues throughout our body. The proteins of the ECM are multifunctional aiding cell migration, morphogenesis, proliferation, differentiation and apoptosis [69]. A range of cell receptors, particularly the integrins, helps the ECM proteins to influence the above functions. Along with the ligand-receptor interactions mediated by the ECM, the surface topography and matrix substrate geometry can also control the cell adhesion receptors and initiate a range of responses [70]. Reports exist that electron beam soft lithography induced surfaces with pits have differential effect on bone marrow stromal cells (BMSC) differentiation. The disordered structure of the pits led to osteogenic differentiation whereas the ordered pit structure retained the multipotency without any spontaneous differentiation of the BMSC [70–72]. The same team reported that if the surface topography is disordered it can induce osteogenic progenitors in embryonic stem (ES) cells [73]. These studies showed that the role of surface topography can modulate the differentiation capacity of stem cells. Before proceeding for differentiation, it becomes important to know the role of ECM surface towards the growth of stem cells.

It has been found by researchers that the complexity of microenvironment and matrix stiffness can regulate the activity of breast cancer cells grown *in vitro* in 3 dimensional alginate hydrogel [74]. The tumour cells are stiffer than the normal cell because the ECM is remodeled by the resident fibroblasts and also by the enhancement of transformed epithelium contractibility. The growth factors and chemokines secreted by the tumour cells further induce inflammation which modifies the collection of the T lymphocytes thereby activating the stromal fibroblasts. These stromal fibroblasts get transdifferentiated into myofibroblasts, aggravating and inducing tissue desmoplasia. The myofibroblasts then deposit a large amount of ECM proteins, secretes growth factors and produces strong contractions on ECM. Consequently, the freshly deposited and remodeled fibres of collagen and elastin get reoriented, cross linked by LOX and transglutaminase giving rise to large and highly rigid fibrils, thereby stiffening the tissue [38]. The ECM from cancer cells can thus provide a rigid support and may contain some residual growth factors that can alter the growth rate of stem cells.

In this review we have discussed about the composition of the ECM in details. The different proteins and non-proteoglycan carbohydrates that compose the ECM has been discussed. The flexibility of the ECM and their dependence on the microenvironment was also mentioned. Finally the role of ECM in modulating cancer has been discussed citing examples of different types of cancer. The review informs the authors about the ECM remodeling that happens in cancer microenvironment.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare no conflicts of interest.

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