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REVIEW: STIFFENING OF THE EXTRACELULLAR MATRIX IN CANCER

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Abstract

Cancer cells enhance tumour progression by remodelling the tumour microenvironment, including stiffening of the extracellular matrix (ECM). The composition and the quantity of the ECM components determine predominantly the ECM stiffness. In cancer cells the ECM is modified, by altered expression of ECM components and extensive cross linking. A stiffened ECM affects critical biological processes by enhancing cancer development, for example increases in proliferation and mobility of cancer cells, induction of abnormal angiogenesis, drug resistance and immunosuppression. Identifying the mechanisms that contribute to ECM stiffening is therefore of great importance to develop novel therapeutic strategies for preventing further malignant transformation. In this review, the ECM components contributing to ECM stiffening are discussed, even as the mechanisms behind ECM stiffening, the biological effects of ECM stiffening and therapeutic strategies.

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Introduction

Cancer is a major cause of death in every country worldwide (Bray et al., 2021) and the number of cancer incidences is expanding worldwide at a fast pace (Fu et al., 2016). Therefore, cancer is a serious threat to public health (Huang et al., 2021). Cancer cells modify the tumour microenvironment (TME) for stimulating tumour growth and progression. One of the known biophysical modified characteristics of TME is stiffening of the extracellular matrix (ECM) (Gonzalez et al., 2019). ECM is composed of the interstitial matrix and the basement membrane, either having their specific function, composition and location. The interstitial matrix provides architectural support in tissues and organs by interconnecting cells in the stroma and by connecting to the basement membrane, forming a three-dimensional network. Collagen and fibronectin, the main components of the interstitial matrix are synthesized and secreted by the surrounding cells of each tissue and organ. Therefore, the composition of the interstitial matrix can vary between different tissue types (Mouw et al., 2014). The basement membrane is a pericellular matrix type, it is a sheet-like dense structure found between parenchyma and connective tissue holding parenchymal cells together and dividing tissues in well-defined compartments. The major components of the basement membrane are collagen and laminin (Lebleu et al., 2007; Halfter et al., 2015). Collagen present at the stromal side provides structural stability, whereas laminin localized at the epithelial side presents many epithelial cell adhesion sides (Paulsson, 1992). Through these sides ECM surface receptors, such as integrins and Hyaluronan acid (HA) receptor CD44, can interact with epithelial cells. Thereby regulating cellular processes, including proliferation, migration, invasion, survival, apoptosis and differentiation (Yamada et al., 2011). The epithelial cells can regulate in turn the ECM by rebuilding and remodelling the ECM through synthesis, degradation and chemical modifications (Lu et al., 2011). This shows that there is reciprocal interaction between ECM and the cells to maintain tissue homeostasis. As well, the ECM adapts constantly through cell-matrix adhesion to changes in the microenvironment, such as mechanical tension, compression and shear. The adhered cells to the ECM sense the mechanical stress by actively pulling on integrins, which provide information about the softness or stiffness of the ECM. The mechanical cues are transduced into chemical signals, stimulating intracellular signalling pathways in order to induce specific changes in gene expression which regulate cell shape, survival, invasion and migration (Bershadsky et al., 2006; Chiquet et al., 2007). However, the triggered signalling pathway by mechanical stimuli may differ between tissue types. One of the adhered cells to the ECM are fibroblasts, they produce ECM components as a response to increased tensile stress. Resulting in increased ECM deposition which contributes among others to ECM stiffening (Desmoulière et al., 2005). In general, stiffness is a characteristic of material, which indicates to what extent the material offers resistance to deformation in response to applied forces (Roylance, 2008). Stiffness in the ECM can be measured through active microrheology (aAMR) which probes microbeads by using optical forces and subsequently measures the response of the surrounding ECM (Botvinick et al., 2014). Stiffness is quantified by the SI derived unit of pressure, Pascal (Pa). However, there is no strict value that indicates whether a tissue is stiffened, this is due to the variable range between tissue types. A stiffened ECM is known to enhance malignant phenotypes, for example increased proliferation and mobility of cancer cells, abnormal angiogenesis, drug resistance and immunosuppression (Huang et al., 2021).

A comprehensive understanding of cancer cells and the supportive tumour environment for malignant behaviour is of great importance to discover novel therapeutic strategies for cancer treatment. In this review is the focus on ECM stiffening in the TME, wherein the major components of the ECM which play a role in ECM stiffening is primarily discussed. Secondly, the mechanisms behind ECM stiffening and subsequently the effects of a stiffened ECM on biological processes, including proliferation, metastasis, angiogenesis, drug resistance and immunosuppression are reviewed. As last, therapeutic strategies for targeting ECM stiffening are considered.

ECM composition in cancer tissue

One of the factors that contribute to ECM stiffening is increased ECM deposition. The production of ECM components is among others regulated by fibroblasts, they produce structural macromolecules and the secretion of enzymes involved in the modification and degradation of these macromolecules. The activity of fibroblasts seems to be upregulated in cancer tissue and therefore also the production of macromolecules, such as collagen, fibronectin and HA (figure 1).
Collagen is the main component of the ECM in healthy tissue and even more abundant in cancer tissue. During collagen maturation the collagen fibers are intermolecular cross-linked by an enzyme called lysyl oxidase (LOX) (Tjin et al., 2017). LOX can crosslink collagens and integrins through oxidation of peptidyl lysine into peptidyl α-aminoad- ipic-δ-semialdehyde. The formed peptidyl aldehydes can condense with each other forming the covalent cross linkages (Kagan et al., 1986). The formed cross linkages determine the elasticity and stiffness of the ECM, increased collagen crosslinking is another feature of ECM stiffening.
Collagen crosslinking is also mediated by the insoluble form of fibronectin that is also produced by fibroblasts. Therefore, fibronectin plays an important role in reinforcing the ECM (Kadler et al., 2008). Besides that, fibronectin also exert regulatory functions by interacting with other proteins, such as integrins and various growth factors and thereby enhancing invasion and migration (Zollinger et al., 2017; Zhu et al., 2014; Dallas et al., 2005). Therefore, increased fibroblast deposition might play a role in malignant transformation.
HA determines the elasto viscosity of the ECM and interacts with other cells through membrane receptors, including CD44 and receptor for hyaluronan-mediated motility (RHAMM). These membrane receptors appear to be overexpressed in lung cancer cells compared to normal cells (Resnick et al., 1998; Yang et al., 1994). In normal biological processes CD44 plays a vital role in aggregation and migration, however in cancer cells it is involved in tumour invasion and metastasis (Sironen et al., 2011; Sneath et al, 1998). RHAMM is normally poorly expressed in regular tissue and mediates cell proliferation and migration, nonetheless in prostate cancer cells is RHAMM expression upregulated and associated with metastasis (Telmer et al., 2011; Gust et al., 2009).
This all is summarized in figure 1, which is a schematic representation of the ECM composition given in normal tissue and in cancer tissue wherein the ECM is stiffened. It is shown that collagen is overabundant and extensively crosslinked in cancer tissue, together with an overexpression of fibroblasts, fibronectin, HA and other ECM components. This indicates that the balance between ECM components is crucial for tissue homeostasis, even as the ECM organization. When tissue homeostasis is dysregulated, ECM stiffness might develop.

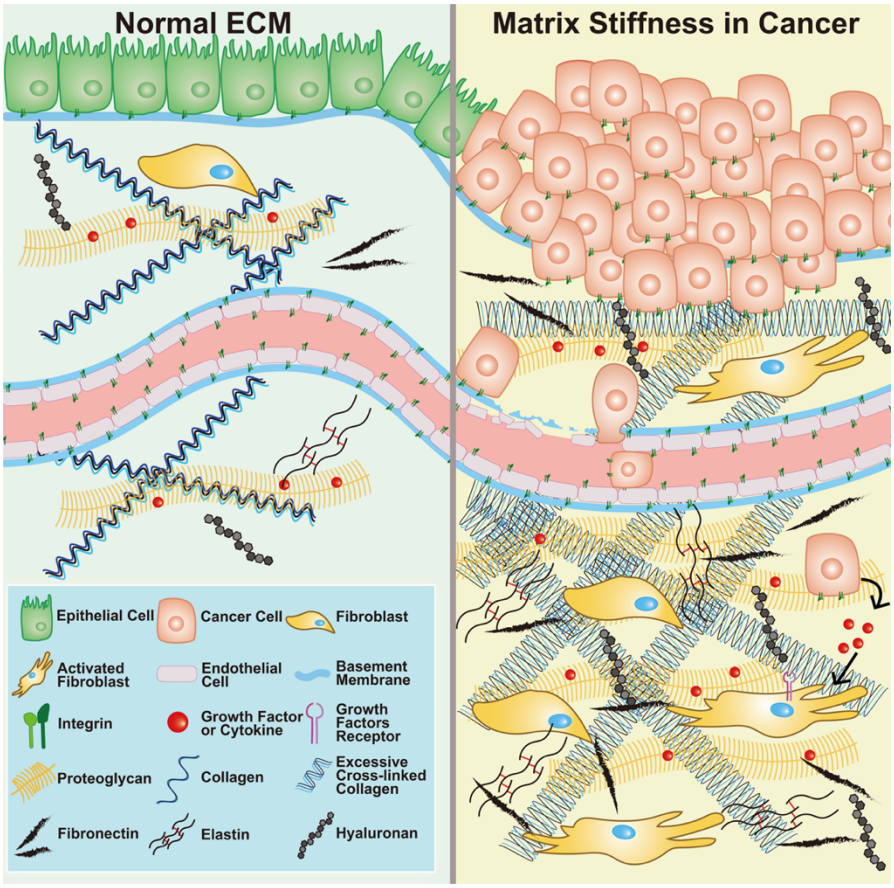

Figure 1 Schematic figure of ECM components in normal tissue (left) and tumour microenvironment (right). From "Extracellular matrix and its therapeutic potential for cancer treatment", by J. Huang et al., 2021, Signal Transduction and Targeted Therapy 6, 153.

ECM stiffness mechanisms in cancer

As discussed earlier, stiffening of the ECM is among others determined by the quantity and the components of the ECM. Although any adhered cells to the ECM can produce ECM components, some factors are associated with the elevated deposition of the ECM components, including tumours, transforming growth factor-β (TGF-β) and cancer-associated fibroblast (CAFs).

As obvious, the increased deposition of the ECM components can be associated with higher production rate in combination with a lower catabolism. One of the causative factors for higher production rate are tumours, they are also able to synthesize components of the ECM. For example, lung cancer cells and oesophageal cancer cells are able to produce collagen and high levels of HA is among others found in breast cancer cells, prostate cancer cells and epithelial ovarian cancer cells (Fang et al., 2019; Anttila et al., 2000; Auvinen et al., 2000; Lipponen et al., 2001). The synthesis of ECM components is in general stimulated by TGF-β, which induces the expression of collagen and fibronectin by activating the canonical SMAD pathway through binding to its serine/threonine kinase receptor (Biernacka et al., 2011). Besides that, TGF-β also stimulates the activation of fibroblasts. Normally, fibroblasts are quiescent and solely activated during wound healing (Gabianna et al., 1971). Once activated by TGF-β, the fibroblasts exert physical forces and increase the synthesis of ECM components to modify the ECM (Tomasek et al., 2002; Parsonage et al., 2005). In non-small lung cancer, high levels of TGF-β are found in the TME correlating with poor prognosis (Hasegawa et al., 2001). Due to the over stimulation of fibroblast in cancer, fibroblasts act as a synthesis machinery producing ECM components. These fibroblasts are called cancer-associated fibroblast (CAFs). Especially, collagen production is elevated by CAFs this is due to the upregulation of collagen genes in CAFs found in human breast carcinomas (Bauer et al., 2010). In addition, CAFs release enzymes, including LOX and matrix metalloproteinase (MMP) that cross link and degrade the ECM respectively, thereby remodelling the ECM as well.

Modifications in the ECM are sensed by cancer cells through mechanoreceptors, such as p130 CAS, growth factors receptors, and integrins. These sensors transmit the chemical cues by recruiting multiple adhesion molecules, one of them is Rho-GTPase. It promotes actin polymerization and actomyosin contraction through Rho effector mDia1 and by stimulating Rho-dependent kinase ROCK I/II respectively. This results in the generation of traction forces around the cancer cells causing a positive feedback loop to enhance EMC stiffness. Besides Rho-GTPase, other adhesion molecules are recruited, including FAK, SRC and RAS GTPases which activate a signalling cascade that regulates gene expression involved in cell shape, survival, invasion and migration (Jaffe et al., 2005; Butcher et al., 2009; Northey et al., 2017; Samuel et al., 2011). In this way, ECM stiffness enhances ECM stiffening that has pro-tumour effects and thereby enhancing tumour progression.

The biological effects of ECM stiffness

A stiffened ECM has diverse effects on biological processes in cancer cells to enhance tumorigenesis and tumour progression, for instance increased proliferation, metastasis, angiogenesis, drug resistance and immunosuppression (Huang et al., 2021). The effects on the biological processes will be discussed in this chapter.

Proliferation

An increased proliferation rate is observed in tumour cells. Tumour cells can manage this through various ways, including stimulating the cell cycle progression by itself and escaping the cell cycle suppression checkpoints.

In 'normal' cells, the cell-division cycle is tightly regulated by the G1/S cell cycle checkpoint. For cell cycle progression is cellular adhesion to the ECM needed, the adhesion allows Ras activation which is mediated by growth factor ligation. The activated Ras stimulates the activation and the translocation of Erk to the nucleus and activates cyclin D1 to form complexes with cyclin-dependent kinases, CKD1 and CDK4, driving cell cycle progression (Schwartz et al., 2001; Xiong et al., 2013; Keely, 2011). Studies has shown that 3D malignant mammary epithelial cells are able to increase cell proliferation by producing their own ECM ligands, enabling the cascade activation for cell cycle progression (Zahir et al., 2003).

Another way to elevate cell proliferation is by escaping the suppression of the cell cycle checkpoints. Under normal conditions, TGF-β activates p53 and stimulates the phosphorylation of Smad enhancing p21 and p27 that inhibit CDK's, and therefore inhibiting cell proliferation. However, this signalling pathway can be interrupted by p130cas that inhibits the phosphorylation of Smad and thereby reducing the expression of cell cycle inhibitors e.g., p21. This enables cells to evade the checkpoint suppression (Kim et al., 2008). In human cancer cells, in particular breast cancer cells and melanomas is an overexpression of p130cas found which contributes to increased cell proliferation by suppressing the antiproliferative cues induced by TGF-β (Kim et al., 2008).

When the proliferation of tumour cells is increased, the tumour mass expands and thereby exerts physical forces on the host tissue. However, the host tissue also generates physical forces on the tumour, hindering the outgrowth of the tumour (Malanchi, 2013). To overcome the stress created by the host tissue, tumour cells modify their TME by stiffening and reorganizing the ECM (Lu et al., 2012). Whereas a stiffened ECM in turn increases the proliferation rate, showing a positive feedback loop.

Stiffening of the ECM increase the proliferation rate through multiple mechanisms. A study did research in hepatocellular carcinoma (HCC) cells and showed that a stiffer ECM enhances significantly the proliferation of HCC cells mediated by the regulation of Erk (Zhang et al., 2017). Stiffened ECM appears to enhance the activity of Erk and thereby the activity of cyclin D1 accelerating the cell cycle progression. In addition, stiffening of the ECM suppresses the Hippo pathway, as shown in figure 2. In human breast, prostate and colon cancer it starts with the activation of integrin linked kinase (ILK) which increases the phosphorylation and thereby inhibiting the activity of myosin phosphatase target subunit 1. Which in turn suppress diverse signalling components of the Hippo pathway, including Merlin, MST1/2, and LATS1/2 (Serrano et al., 2013). The inhibition cascade causes the translocation of YAP/TAZ into the nucleus, where it can activate the transcription of particular genes participating in cell proliferation, such as cyclin D1 (Sabra et al., 2017; Mizuno et al., 2012). When ILK is inhibited, the Hippo pathway is activated, YAP/TAZ remains in the cytoplasm

and tumour cell growth and survival is suppressed (Serrano et al., 2013). This finding confirms the involvement of the Hippo pathway in tumour progression. The mechanical activation of YAP allows tumour cells to escape the checkpoint suppression and to obtain an uncontrolled proliferation (Zhao et al., 2007; Dupont et al., 2011).

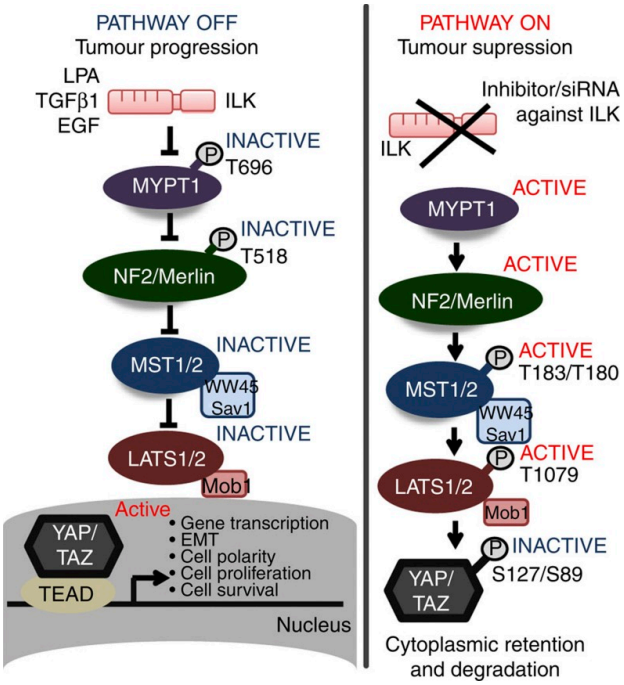

Figure 2. Schematic representation of the Hippo pathway regulated by ILK. From "Inactivation of the Hippo tumour suppressor pathway by integrin-linked kinase", by Serrano et al., 2013, Nature communications, 4, 2976.

Invasion and metastasis

For metastasis is the intravasation of cancer cells into the blood or lymphatic circulation necessary so it can spread from the primary tumour site through the whole body and might extravasate causing a tumour colony at a secondary site (Celià-Terrassa et al., 2012). However, during tumorigenesis and tumour progression, collagen deposition increases, linearizes and stiffen. It was first thought that for tumour invasion and migration the reorientated collagen and the increased density must be degraded. Nonetheless, the conformation of collagen is so altered that the cleavage binding site is hidden for MMPs, preventing degradation of the ECM (Fang et al., 2014). Thus, the increased collagen deposition which hinder the entrance of MMP may act as a defence mechanism for tumour invasion.
Although collagen degradation is believed to be a prerequisite for tumour invasion, a stiffened ECM appears to have beneficial effects on the mobility of cancer cells, the stiffened collagen acts as an invasion 'highway' for cancer cells. Through multiple pathways is the stiffened ECM able to regulate cell migration. The induced bundling of collagen by integrins

enhances the gathering of adhesion molecules, for instance Ras, Rho and Rac. Rho promotes ameboid migration, whereas Rac enhances mesenchymal migration (Sanz-Moreno et al., 2008). The activity of Rho is regulated by Ras and tumour suppressor p53, which stimulate and inhibit the activity respectively (Chen et al., 2003; Xia et al., 2007). Ameboid migration is characterized by no breakdown of the ECM but the cells change their shape and press themselves together to fit through gaps in the ECM. Whereas mesenchymal migration shows proteolytic remodelling of the ECM by adhering to the ECM through integrins and generating miniscule pores in the ECM by the proteases where the cells can migrate through (Friedl et al., 2010).
The statement that a stiffened ECM has beneficial effects on the mobility of cancer cells is supported by findings in the respective of LOX. Studies have shown that overexpression of LOX enhances tumour progression and metastasis particularly in lung cancer and breast cancer (Erler et al., 2006; Gao et al., 2010). When LOXL2 expression is induced in non-invasive breast cancer cells, tumour fibrosis and tumour invasiveness is enhanced (Akiri et al., 2003). Whereas in lung cancer mouse model, a reduction in LOX expression showed a decrease in collagen cross linking and a hindered tumour progression (Gao et al., 2010).

Angiogenesis

Another term for angiogenesis is neovascularization and is the process of linking new capillaries to pre-existing blood vessels in nutrient-deprived tissues. Those nutrient-deprived tissues are frequently found in regions adjacent to tumours, presumably due to the additional required supply of oxygen and nutrients to tumours for growth and survival (Bergers et al., 2003). The required supply is facilitated by angiogenesis that is stimulated by pro-angiogenic factors which are secreted by the ECM (Zhou et al., 2009). However, the ECM also contains anti-angiogenic factors indicating the dual role of ECM in angiogenesis. Human breast cancer cells and cervical cancer cells are able to disrupt the balance between the secretion of pro- and anti-angiogenic factors and thereby creating an angiogenic phenotype (Hanahan et al., 1996). Overproduction of pro-angiogenic factors results in a disorganized vessel network in tumours because the newly formed vessels cannot mature and stabilize (Mcdonald et al., 2005). One of the pro-angiogenic factors is vascular endothelial growth factor (VEGF) which promotes the proliferation and migration of vascular endothelial cells and induces the branching of endothelial cells from the pre-existing vessels (Dimmeler et al., 2000; Fernandez et al., 1999). The production of VEGF is among others determined by the concentration of oxygen in surrounding tissues and oxygen concentration can be in turn affected by ECM stiffening that is known to induce a hypoxic environment. Hypoxia enhances the binding of hypoxia inducible factors (HIFs) to cis elements in the VEGF promotor, stimulating VEGF expression and thereby promoting angiogenesis (Liu et al., 1995; Tang et al., 2004). Besides that, HIF also appears to regulate LOXL2 that through collagen cross linking in the basement membrane regulate the spreading of vascular endothelial cells in zebrafish (Erler et al., 2006; Bignon et al., 2011).
ECM stiffening is also known to regulate the expression of VEGF in HCC cells. In addition, ECM stiffening upregulates the expression of VEGF receptors in vascular endothelial cells enhancing the ability to sense VEGF. VEGF signals are sensed and transduced by VEGF receptors on vascular endothelial cells, the primary VEGF receptor is VEGFR2 and plays an important role in angiogenesis. Research indicated a positive correlation between VEGFR2

expression and increased ECM stiffness. This is based on the increasing expression of VEGFR2 in Human Umbilical Vein Endothelial Cells (HUVECs) when grown on higher stiffness substrates (Wang et al., 2020).

Drug resistance

The disorganized vascular network in tumours caused by the overproduction of pro-angiogenic factors has an impact on drug distribution. Drugs are mainly distributed through diffusion, however solid tumours contain already a low number of micro vessels making it harder for the drugs to get access to the tumour site (Offersen et al., 1998; da Silva et al., 2009). Stiffening of the ECM reduces the drug diffusion even more due to compressing and penetrating the micro vessels causing a vascular leakage and thereby impairing the transport of drugs (Ramjiawan et al., 2017). In addition, a stiffened ECM hinders drug diffusivity by forming a drug infiltration barrier between vessels and the tumour. This all contributes to the development of drug resistance.

Immunosuppression

Surveillance of the immune system is of great importance to prevent tumour formation. However, during cancer development the immune cells become regulated by the ECM and enhance the exertion of pro-tumour roles. The focus will mainly rely on T cells in this paragraph.
The altered ECM and increased density of collagen have an impact on immune functioning. Normally T cells migrate towards the tumour through chemotaxis gradients, however these gradients are disturbed by the modified ECM. Resulting in migration along the cancerous tissue instead of entering them (Luo et al., 2020; Ferrero et al., 1998). Hypoxia induced by ECM stiffening stimulates TGF-β production that facilitate the differentiation of regulatory T (Treg) cells, which exhibit pro-tumour effects (Castermans et al., 2007). Besides a hypoxic TME, the environment is also often acidic due to impaired exchange of substances caused by poor diffusion. The acidification of the TME decreases T cell activity through co-interaction of immunoglobulin T cell suppressor and an inhibitory ligand present in the acidic TME (Johnston et al., 2019). The activity of T cells is also suppressed by collagen, in particular CD8+ T cells. The suppression is mediated by the activation of leukocyte-associated Ig-like receptors (LAIRs) which are found in high quantities on immune cells. LAIRs contain immunoreceptor tyrosine-based inhibition motifs (ITIMs) and these motifs enable the suppression of T cell activity (Roth et al., 2006; Meyaard, 2008). Furthermore, CD8+ T cell activity can also be inhibited by PD-L1, whose expression is upregulated in lung cancer cells due to stiffening of the ECM (Miyazawa et al., 2018).
This all indicates, ECM stiffness has indirect a negative impact on anti-tumour effects of T cells.

Therapeutic strategies

Due to the fact that ECM stiffness is a hallmark of cancer, targeting the stiffened ECM might be a potential treatment for cancer. Diverse mechanisms contribute to stiffening of the ECM and therefore multiple options might exist to target ECM stiffening.

In the previous chapter is the drug infiltration barrier induced by ECM stiffening described. For enhancing drug sensitivity, it might be beneficial to break down collagen so anti-cancer drugs can penetrate into tumour tissue. One way to do so might be using collagenases, which depletes collagen by breaking the peptides bond in collagen down. Collagenases are already used in the clinic for patients with severe backpain due to massive accumulation of collagen (Sussman et al., 1981). However, using collagenases in the context of cancer treatment is questionable. Treatment with collagenase shows an increase in molecule diffusion, nonetheless the increase is only modest. Therefore, it is uncertain if this improvement is satisfactory for therapeutic levels and taking into account degradation of the ECM benefits also tumour metastasis and affects healthy tissue (Dolor et al., 2018). Another therapeutic target for diminishing collagen might be TGF-β, which regulates the expression of collagen. Research has shown that TGF-β is inhibited by losartan when it is intratumorally and intravenously administered. Losartan is an angiotensin ll inhibitor and mediates the suppression of TGF-β by reducing the activity of angiotensin II type I receptor (AGTR1) that activates TGF-β (Diop-Frimpong et al., 2011). Treatment with losartan improves drug penetration of nanoparticles. Besides, losartan is also already used in the clinic to control hypertension and limited safety risks have been reported compared to collagenases. However, TGF-β plays a dual role in cancer development. In the initial stages of cancer, it acts as a tumour suppressor by inhibiting tumour cell growth, whereas in the later stages it has pro-tumour effects by stimulating Treg differentiation and synthesis of ECM components inducing an immunosuppressive tumour microenvironment (Chen et al., 2020). Therefore, the application of TGF-β treatment should be taken with caution.
Furthermore, LOX might be a potential target due to its involvement in stiffening of the ECM by crosslinking collagens. LOX can be targeted in various ways, for example with the use of antibodies or using competitive inhibitors. Study with human tumours biopsies has shown that an antibody treatment against LOXL2 reduces the number of collagen cross linkages, diminishes the activity of fibroblast and thereby affects the synthesis of growth factors (Barry-hamilton et al., 2010). Nonetheless, this treatment seems only to be effective in the early stages of cancer and not in the advanced stages. This might be due to the highly dense ECM in the advanced stages (Benson et al., 2017). Another way to target LOX is by the use of a competitive inhibitor β-aminopropionitrile (BAPN) that disrupt collagen crosslinking severely. In addition, metastasis is also affected by BAPN. Treatment with BAPN decrease metastasis development in mice significantly. However, this was only observed when BPAN was administered before tumour cell injection and not after. Indicating this treatment is most effective in the initial stages of extravasation (Alla et al., 2009).
Developing other effective LOX inhibitors is not that convenient because the complete mammalian LOX structure is not discovered yet (Cox et al., 2016). Consequently, there is thought of other mechanisms to affect LOX, for example depleting the catalytic site of LOX. Treatment with tetrathiomolybdate chelates copper, which is a key component of LOX. This treatment was first set up for patients with Wilson disease (Brewer et al., 2009). However, research indicated that tetrathiomolybdate treatment is also effective and safe in breast

cancer patients with a high relapse chance. Depletion of copper induced by tetrathiomolybdate is associated with a reduction in LOXL2 serum levels (Chan et al., 2016). Thereby the remodelling of the ECM is impaired, rendering the potential for tumour progression.

Discussion

Cancer cells demand to create a surrounding environment that is beneficial for tumour growth and progression. This is among others achieved by remodelling the components of the TME, one of the components is the ECM. A well-known remodelled characteristic of the TME is stiffening of the ECM (Gonzalez et al., 2019). A stiffened ECM is among others caused by increased deposition of ECM components, such as collagen, fibronectin and HA, and extensive crosslinking of ECM components induced by LOX. In general, the synthesis of ECM components is mediated by TGF-β through activating the SMAD pathway. In non-small lung cancer are high levels of TGF-β found that also activates CAFs and thereby stimulates ECM components synthesis (Hasegawa et al., 2001). The induced modifications in the ECM are sensed by cancer cells through mechanoreceptors, consequently transduced into signals and generates traction forces around cancer cells and regulates gene expression involved in cell shape, survival, invasion and migration (Butcher et al., 2009; Northey et al., 2017). In this way, ECM stiffness enhances ECM stiffening that has promoting effects on cancer development, for instance increased proliferation, metastasis, angiogenesis and drug resistance (Huang et al., 2021).

Stiffening of the ECM enhances the proliferation of HCC cells by activating Erk and thereby cyclin D1, accelerating cell cycle progression (Zhang et al., 2017). Besides, ECM stiffening also stimulates the expression of genes participating in cell proliferation through acting on the Hippo pathway.

In tumour invasion and metastasis plays the ECM stiffening a dual role. First it was thought that the increased collagen deposition must be degraded before tumour cells are able to invade surrounding tissue and migrate. However, a stiffened ECM hinders the entrance of MMP and thereby prevents degradation and hinders indirect invasion and migration, acting as a tumour defence mechanism (Fang et al., 2014). Nonetheless, a stiffened ECM also appears to exert beneficial effects on the mobility of cancer cells through acting on multiple pathways, i.e., Ras. The promoting effects of a stiffened ECM on mobility are also supported by findings in the context of LOX. Overexpression of LOX induces invasion in non-invasive breast cancer cells whereas a reduction hinders tumour progression (Akiri et al., 2003; Gao et al., 2010).

Furthermore, stiffening of the ECM can induce a hypoxic TME that stimulates the expression of pro-angiogenic VEGF and thereby promoting angiogenesis. Besides, a stiffened ECM also upregulates the expression of VEGFR2 thereby enhancing the ability to sense VEGF (Wang et al., 2020). However, overproduction of VEGF results in a disorganized vessel network in tumours because the newly formed vessels cannot mature and stabilize (Mcdonald et al., 2005). This has also an impact on drug distribution because drugs are mainly distributed through diffusion. A stiffened ECM reduces drug diffusion even more by compressing the micro vessels and hinders drug diffusivity by forming a drug infiltration barrier, initiating drug resistance.

In addition to drug resistance, a stiffened ECM also suppresses the immune system in multiple ways. The altered ECM disturbs the migration of T cells into cancerous tissue, facilitates the differentiation of regulatory T cells and decreases T cell activity contributing to anti-tumour effects of T cells (Luo et al., 2020; Ferrero et al., 1998; Castermans et al., 2007; Johnston et al., 2019).

All these findings indicate that ECM stiffness is a hallmark of cancer and contribute to tumour progression. Cancer incidences are expanding worldwide and is a serious threat to public health. Therefore, it is of great importance to develop novel therapeutic strategies and targeting stiffening of the ECM might be a potential cancer treatment.
Stiffening of the ECM can be induced by multiple mechanisms, therefore various possibilities might exist to target ECM stiffening.
An obvious treatment might be the use of collagenases, which degrade the stiffened ECM and thereby enhance the drug sensitivity of other anticancer drugs modestly. However, the degradation also affects healthy tissue and is beneficial for tumour metastasis (Dolor et al., 2018). Another potential target might be TGF-β, which regulates the expression of collagen. TGF-β can be inhibited by losartan, however the treatment may only be used in the later stages of cancer due to the dual roles of TGF-β during cancer development (Chen et al., 2020). Furthermore, targeting LOX might be a potential treatment by inhibiting the cross linking of collagen via antibodies or competitive inhibitors, such as BPAN. Nonetheless, both treatment options seem to have limited efficacy. So far, the treatments are modestly effective or they exert some limitations. Although tetrathiomolybdate treatment, which deplete the catalytic site of LOX seems to be effective and to my knowledge no major limitations have been reported yet, therefore it can be considered as a safe treatment (Chan et al., 2016).

To summarize, stiffening of the ECM is a distinctive feature of cancer and modifies biological processes to enhance cancer development. Targeting ECM stiffness as a therapeutic strategy for cancer is more complicated as it seems due to limitations and side effects. Nonetheless, tetrathiomolybdate seems to be a potential treatment for impairing tumour progression by affecting the ECM through LOX.

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