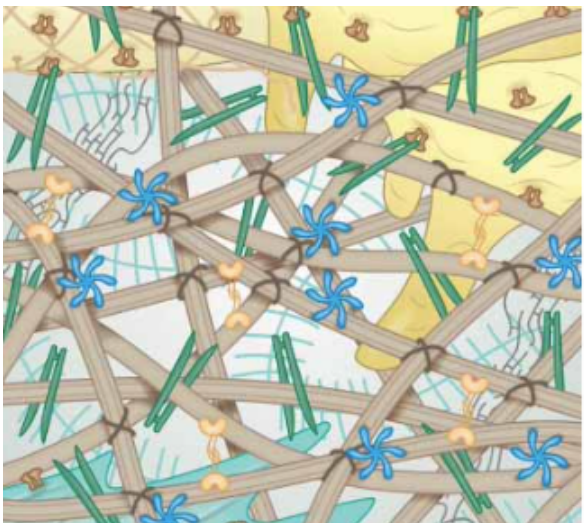
Extracellular matrix alterations that might increase the risk for COPD patients to develop lung cancer



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Foreword

It is of great importance for Biology bachelor students to be able to carry out a literature research at an academic level based on a problem definition, goal and research question. For this reason, I conducted a literature research for four weeks under supervision of doctor Corry-Anke Brandsma. She is specialized in the pathophysiology of Chronic Obstructive Pulmonary Disease (COPD) among others. After several meetings, the subject of the thesis was defined: extracellular matrix (ECM) alterations found in COPD that could also be attributed to cancer development. This field of interest is very important as it may help to prevent the development of cancer in patients with COPD.

In this thesis, I will first give an overview of the architecture, components and functions of the ECM in the lung. Additionally, I will give some background on COPD and lung cancer. Then, I will focus on the changes that can be seen in the ECM of patients with COPD and lung cancer and if there are resemblances between the ECM changes in these two pathologies. Lastly, I will try to give an explanation on how these ECM changes in COPD might contribute to the development of cancer. I hope that this thesis will contribute to the existing knowledge in the field and that it will spark interest to the readers.

Rosalie
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Summary

Two pathologies that are known to have a high mortality are Chronic Obstructive Pulmonary Disease (COPD) and lung cancer as they are the fourth cause of death in the world[2] and the first cause of cancer mortality in the world[3], respectively. Both pathologies share common risk factors, including smoking[2,4]. It has been shown that patients with COPD have an increased risk to develop lung cancer[2]. Current data show that the extracellular matrix (ECM) composition and architecture changes with certain disease progressions[5]. These changes might precede modifications in cell behavior and therefore actively contribute to the onset of diseases[6], as could be the case for COPD and lung cancer. Additionally, ECM alterations observed in COPD might contribute to the onset of cancer. However, the process by which the ECM might be involved remains unclear. Therefore, this thesis will give an overview of what has been found in previous research on ECM alterations in COPD and lung cancer, show which ECM alterations were found in both pathologies and explain how these ECM alterations might contribute to the onset of cancer. The thesis will specifically focus on the core matrisome.

Core matrisome alterations that were found in both pathologies are a general increase and similar remodeling processes of collagen and increased osteopontin, fibulin-5, fibronectin, tenascin C and hyaluronan levels. While several of these ECM components were found to be involved in one or multiple hallmarks of the onset of cancer, others were only involved in processes that occur once the mature and/or metastatic tumor has already been formed. ECM alterations were also found that were not involved in cancer development, but conversely, in defense mechanisms to suppress cancer metastasis.

Although there are some hypotheses on how ECM changes can increase the risk to develop lung cancer, more research in humans with bigger sample size and research that also includes patients with both pathologies are needed to confirm whether these hypotheses are indeed causal to cancer development.

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Introduction

The extracellular matrix (ECM) is a macromolecular framework that is tissue-specific[6]. It consists of noncellular aspects of the tissue such as fibrous proteins, glycoproteins and proteoglycans that altogether form a highly dynamic complex that provides physical and structural support of the cells and thereby determines the lung's tissue architecture[5]. Depending on its tissue localization and physiological conditions, the ECM composition may vary[6].

The ECM is crucial for the normal functioning of the lung. It can shape cellular behavior by delivering spatial, contextual, and biomechanical cues to cells[5, 6]. As a consequence, morphogenesis during development and homeostasis are directed. Furthermore, extracellular substrates can interact with integrins on the surface of cells[1]. Integrins are signal molecules and membrane receptors that form the link between the ECM and the cytoskeleton. Upon binding with extracellular substrates, the cell reacts to the stiffness of the substrate which can result in changes of cell morphology, activity, gene expression and in cell differentiation[1, 5]. Features of the ECM also provides cues to guide cells to their position and the microenvironment in which they function[6]. These processes makes proper functioning of tissue-specific differentiated cell types possible as well as the regulation of stemness and differentiation of stem cell populations[1].

The ECM of the lung consists of two main structures: the basement membranes and the interstitial spaces[1]. The basement membranes are thin layers of glycoproteins that are located under the cell layers of epithelia and endothelia[6]. They also cover peripheral nerve cells, muscle and fat. The interstitial matrices mainly consists of collagen and elastic fibers and forms a fibril-like meshwork that maintains cohesiveness and biochemical characteristics of the lung through interconnections between cells within the tissue[1]. Collagen provides tensile strength but low elasticity to the lung. Reversely, elastin, one of the main components of elastic fibers, provides the lung with high elasticity and low tensile strength. These characteristics enable compliance and elastic recoil of the lung[1]. Other molecules that are associated with elastic fibers are fibrillins, microfibril associated glycoproteins, fibulins, elastin microfibril interface-located proteins (EMILINs) and members of the lysyl oxidase (LOX) family. Furthermore, the ECM is rich of proteoglycans. These are molecules that contain a core protein component that is covalently linked to glycosaminoglycans or sulfated polysaccharides, which makes them hydrophilic. Consequently, the proteoglycans can form a hydrogel which makes the lung viscoelastic.

The structural and or functional diversity of ECM proteins can be affected by post-translational modifications[1]. Current data show that the ECM composition and architecture also changes with certain disease progressions[5]. Present hypotheses state that these changes might precede modifications in cell behavior and therefore be involved in and even actively contribute to the onset of diseases[6]. Two pathologies in which alterations in ECM composition and architecture have been observed are Chronic Obstructive Pulmonary Disease (COPD)[7, 8] and lung cancer[9, 10].

COPD, as the name implies, is a chronic respiratory disease with high morbidity and mortality. It affects a lot of people as it is the fourth cause of death in the world[2].

It is characterized by airflow limitation, a chronic and abnormal inflammatory response and hyperinflation which occurs when air is trapped in the lung[4]. These processes occur because of a loss of small airways, enlargement of respiratory air spaces in the alveoli

(also called emphysema), loss of elastic recoil and small airway remodeling, such as thickening of airway walls[11].

The main risk factor for COPD is smoking[2, 4]. However, multiple risk factors are found to have an effect, such as exposure to other toxic gasses or particles, genetic predisposition, age and sex[12]. The clinical phenotype of COPD is a result of both genetic factors and exposure, which makes COPD a very heterogenic disease[13]. This heterogeneity can be observed in the clinical characteristics, the progression of the disease, and the response to treatment. Moreover, there is a great difference between patients with respect to the severity and combination of symptoms, such as the progression of emphysema, how much the airways have been affected and how much sputum is being produced[14].

Like COPD, lung cancer also has a high mortality, as it is the first cause of cancer mortality in the world. It accounts for more than 1,400,000 million deaths per year[3, 15].

Lung cancer can be divided in two main groups based on histotype, prognostics and therapeutic implications: small cell lung carcinomas (SCLCs) and non-small cell carcinomas (NSCLCs)[16]. These main groups include multiple types of lung cancer based on WHO classification, such as large cell carcinomas, large cell neuroendocrine carcinomas, squamous cell carcinoma (SqCC), adenocarcinomas, adenosquamous carcinomas, sarcomatoid and pleomorphic carcinomas[17].

The main hallmarks of human cancers are sustained proliferation, evasion of growth suppression, death resistance, replicative immortality, induced angiogenesis, initiation of invasion and metastasis, and dysregulation of cellular energetics and inflammatory processes[18].

Lung cancer development depends on the type of cell from which is originates and the pathway by which it develops[17]. Because of the different processes by which lung cancer can develop, the disease is highly heterogenous in symptoms and disease progression. The majority of cancers are NSCLC, which account for 80% of the cases, while 20% of lung cancers are SCLC[19]. Despite the different processes by which lung cancer can develop, tobacco consumption is the main risk factor for lung cancer. More than 85% of all lung cancer cases occur among current or former smokers[2].

COPD and lung cancer share several important features. They both have a high mortality, are associated with inflammation and they share common risk factors[2]. These include smoking, genetic predisposition and environmental exposures. Although smoking is a main risk factor to develop COPD and lung cancer, some studies have demonstrated that COPD is a risk factor for lung cancer development, independent of smoking exposure[20]. Although data is limited on less common histological subtypes of cancer, it seems that each subtype is linked to COPD[19]. Most importantly, an analysis by De Torres et al. revealed that on average, 16.7 new cases of lung cancer are diagnosed per 1000 individuals with COPD over the course of one year[21].

Multiple ECM changes have been found in patients with COPD as well as in lung cancer. Moreover, several ECM changes were observed in both pathologies[22]. As was mentioned, patients with COPD have an increased risk to develop lung cancer[2]. It is known that the ECM can control cell function and phenotype[1, 5]. Therefore, it might be possible that ECM changes that have been found in both pathologies might be involved in the onset lung cancer. If we understand how ECM changes influence processes involved in disease

progression, and more specifically, how ECM changes in one disease can influence the onset of another, we might be able to improve current therapies and to prevent lung cancer.

Therefore, the aims of this thesis are: 1) to give an overview of what has been found in previous research on ECM alterations in COPD and lung cancer, 2) to show which ECM alterations were found in both pathologies and 3) to explain how these ECM alterations might contribute to the onset of cancer. The changes that will be discussed will be changes in the core matrisome: collagens, glycoproteins and proteoglycans.

Extracellular alterations in Chronic Obstructive Pulmonary Disease

Table 1 summarizes the alterations that were found in COPD. The first major constituent of the ECM in which alterations were observed is collagen. Several researchers found an increased collagen content in the lungs of patients with COPD[23-25]. Besides an increased amount of collagen in the ECM, several remodeling processes were found in patients with COPD including altered fibril morphology[5] and collagen degradation[7].

The second constituent of the ECM in which alterations were observed is in the group of glycoproteins. For instance, a review by Zhou Y, et al. summarized the outcome of multiple researches focused on elastin. These researchers observed major changes in elastin structure and content. For example, an increased elastin content was observed[23, 24]. However, Eurlings IM, et al. and Black PN, et al. found decreased elastin content in small airway walls of COPD patients. Furthermore, elastin remodeling levels were elevated; fragmented elastin was observed and abnormal elastin fibers led to abnormal elastin assembly (e.g. elastin clumps)[23, 24, 28, 29].

Several other glycoproteins seem to be altered in COPD patients as well. For example, the ECM fractional area of fibronectin was found to be higher in small airways of patients with COPD[8]. The same study found that the fractional area of tenascin was higher in both the small and large airways. Furthermore, expression levels of osteopontin, were observed to be higher in the sputum of patients with COPD[30]. Another glycoprotein found to be increased is hyaluronan[1]. Lastly, a genome-wide gene expression profiling of tissue samples of patients with COPD found that the *Fbln5* gene (producing fibulin-5) was one of the most upregulated genes[31].

The amount of proteoglycans were also found to be increased in COPD[5] while the amount of proteoglycans in the alveoli seemed to be decreased[8]. Moreover, synthesis of proteoglycans by central and distal lung fibroblasts is altered in patients with severe COPD[22,32]. A proteoglycan that was observed to be progressively increased upon immune-staining in patients with mild to moderate COPD was versican[29].

So, many alterations in the ECM content have been observed in patients with COPD. These changes include alterations in elastin structure and content, a general increased collagen content, altered fibril morphology, collagen degradation, alterations in the amount of proteoglycans and glycoproteins. To understand if changes of the ECM that are observed in COPD can be causal to the development of cancer, it remains to be examined whether some of these ECM alterations can be observed in cancer patients as well.

ECM alterations in lung cancer

Several studies have been published on ECM changes in lung cancer including several reviews that discuss the available data about the structure and function of the pulmonary ECM in lung cancer[1] and the remodeling processes and post-translational modifications found in lung cancer[33, 34]. Additionally, studies have been performed that used mouse models to characterize the ECM composition in different cancer progression stages[35, 36]. ECM composition of tumor tissue of humans has been studied as well[37, 38]. Lastly, transcriptomic

Table 1: Alterations in the distinct core matrisome constituents in COPD and/or lung cancer described in previous research

Core matrisome constituent	**Alterations in COPD**	**Alterations in lung cancer**	**Source (COPD/lung cancer)**
Collagen	Increased collagen content	Collagen accumulation	[23-25] / [35]
	Altered fibril morphology	Higher level of crosslinked collagens	[5] / [1]
	Collagen degradation		[7]
		Positive correlation of *COL10A1, COL11A1 and COL7A1* with SqCC	[39]
		Higher production of collagen I, III, VI and IV	[37, 38, 40]
Glycoproteins	Increased elastin content		[23,24]
	Decreased elastin content		[26, 27]
	Elevated elastin remodeling levels		[23, 23, 28, 29]
	Higher fibronectin fractionated area	Increased fibronectin levels	[8]/[35, 38]
	Higher tenascin fractionated area	*Tnxb, TncR* included in risk signature Increased tenascin C levels	[8] / [39] [35, 38]
	Higher osteopontin expression levels in spuntum	*Spp1* included in risk signature	[30] / [39]
	Upregulation of *Fbln5*	*Fbln5* included in risk signature	[31] / [39]
		Mfap4, Slit2, Emilin2, Mmrn1 and *Lamb2* included in risk signature	[39]
		Decreased nephronectin and fibrinogens levels and increased laminin levels	[35]
Proteoglycans	Increased versican levels		[29]
	Increased hyaluronan levels	Increased hyaluronan levels	[1]
		Proteoglycan 4 and testican-2 included in risk signature	[39]

and proteomic analysis has been applied to examine the ECM composition in SqCC[39]. The findings of these and several other studies will be discussed in this chapter and can also be found in Table 1.

In general, an extensive stroma of ECM surrounding tumors at primary and metastatic sites is observed in patients with lung cancer[1]. Furthermore, alterations were observed in the amount of collagen and its structure, in the amount of glycoproteins and proteoglycans.

In general, a dramatic accumulation of collagen was found in advanced primary tumors of patients with lung cancer[35]. Parker AL, et al. produced a matrix risk signature including differentially expressed matrisomal genes when comparing tumor and non-tumor tissue of a specific type of cancer (SqCC). Three of them are genes that encode collagens: *COL10A1, COL11A1 and COL7A1.* These genes were all positively correlated with SqCC. The genes that are related to collagen production that were included in the matrix risk signature of Parker AL, et al. are not the only genes that are found to be expressed to a higher degree. These will be discussed later.

Other ECM alterations that were found in patients with lung cancer that are related to collagen production are a higher production of collagen I[40], III[40], VI[37] and IV[38] and a higher level of crosslinked collagens[1]. Higher levels of hydroxylysine aldehyde-derived collagen cross-links (HLCCs) were observed, while the level of lysine aldehyde-derived cross-links (LCCs) was reduced[36].

Another large constituent of the ECM that is affected in patients with lung cancer is the glycoproteins. Several of these proteins are found to be altered in the tumor microenvironment as well. Moreover, most of the genes that were included in the risk signature defined by Parker AL, et al. encode glycoproteins. Examples of these genes are: *Mfap4* (producing microfibril-associated glycoprotein 4), *Slit2* (producing slit homolog 2 protein), *Emilin2* (producing EMILIN-2), *Mmrn1* (producing multimerin 1 or elastin microfibril interfacer 4), *Spp1* (producing osteopontin), *Tnxb* (producing tenascin XB), *TncR* (producing tenascin-R), *Fbln5* (producing fibulin-5) and *Lamb2* (producing laminin subunit beta 2). Other glycoproteins that are associated with lung cancer that are not included in the risk signature are nephronectin, fibrinogens and laminin[35], tenascin C and fibronectin[35, 38]. A study performed by Gocheva, et al. provides an analysis of the matrisome in *in vivo* mouse models of fibrosis and metastatic lung cancer. They found decreased levels of nephronectin and fibrinogens in primary lung tumors. Conversely, increased laminin content was observed. Additionally, they found that tenascin C and fibronectin levels were markedly increased in tumor samples compared to normal lungs. Increased tenascin C and fibronectin levels was also found in a study performed by Sethi T, et al.

Proteoglycan content in the ECM of the lung is also altered in lung cancer. Examples of proteoglycans whose expression is altered are hyaluronan[1], proteoglycan 4[39] and testican-2[39]. Hyaluronan levels were found to be increased in lung cancer. Proteoglycan 4 and testican-2 were both included in the risk signature of Parker AL, et al.

So, the ECM changes that were found to be associated with lung cancer are an extensive stroma in general, an increased amount of collagen and changes in its structure, an upregulation of several proteoglycans while expression of other proteoglycans seem to be reduced, and alterations in the production of glycoproteins.

ECM alterations in COPD and lung cancer and their involvement in the onset of cancer

For cells to become malignant and for cancer to originate, a few processes need to occur. The cell needs to obtain tumorous characteristics that cause the transition from a normal cell to a cancer cell. These characteristics include the ability to survive, grow, and invade[10]. During cancer cell development, the cell eventually loses its ability to differentiate and it changes its microenvironment to promote self-growth. The aim of the thesis is to find explanations on how the ECM changes that are both seen in patients with COPD and lung cancer can attribute to survival, growth, and invasion of normal cells which drives them to become cancer cells.

The first major constituent of the ECM that was included in this thesis was collagen. When comparing the ECM of COPD and lung cancer patients, a general increase and similar remodeling processes of collagen were found. One of the remodeling processes that was found, was a change in the organization of fibrillar collagens. Genes that are involved in the regulation of the collagen fibril architecture are *COL10A1, COL11A1*, and *CTHRC1*. All of these genes were included in the risk signature of Parker AL, et al. The question is how these changes of the fibril architecture can increase the risk of developing lung cancer. In general, an increase in crosslinked and linearized collagens lead to increased stiffening of the tissue. This stiffening upregulates integrin signalling[10] and can therefore directly affect hallmarks of cancer such as proliferation, cell invasion and survival. Additionally, fibrillar collagen architecture has been associated with altered T-cell mediated immune surveillance[41]. For example, it has been suggested that cancer associated fibroblasts that expresses COL11A1, can lead to exclusion of T cells from the tumor microenvironment, thereby contributing to cancer cell survival.

Additionally, it has been found that increased collagen crosslinking and ECM stiffness can promote ERK and PI3 kinase signaling and focal adhesion assembly[42]. ERK and PI3 kinase signaling promotes cell progression and cell division, because the signaling inhibits apoptotic pathways and increases production of anti-apoptotic proteins. The promotion of ERK and PI3 kinase signaling and focal adhesion assembly by increased stiffness of the ECM, facilitates Neu-mediated oncogenic transformation[42]. In this transformation process, normal cells obtain genetic alterations which can lead to uncontrolled growth and might therefore result in the formation of tumors. It is noteworthy that Neu-mediated oncogenic transformation is not always associated with cancer development.

Among the glycoproteins that were differentially expressed in both COPD and lung cancer is osteopontin. The expression level of this protein was observed to be increased in both pathologies. Osteopontin has been identified to stimulate alternatively activated macrophages (M2) polarization[43]. M2 macrophages have been found to be the dominant tumor associated macrophage type in lung cancer[22]. Additionally, M2 macrophages are known to produce factors that contribute to tumor growth and invasion. Also, *in vitro*, tumor associated macrophages induce programmed death-ligand 1 (PD-L1) expression by producing osteopontin. If PD-L1 binds to immune cells, it sends inhibitory signals. Consequently, this leads to the repression of immune cell activity. In this way, osteopontin might contributes to immune evasion mechanisms that enable premalignant progression.

While a lot has been reported about elastin remodeling in COPD, there is not a lot of literature on elastin remodeling in lung cancer. Therefore, no implications can be made on how elastin remodeling might explain a higher risk for lung cancer in COPD patients.

Another glycoprotein that was observed to be upregulated is fibulin-5. A research performed by Yue W, et al. showed fibulin-5 was not involved in tumor progression, but conversely, in suppression of lung cancer invasion[44]. Fibulin-5 suppressed matrix metalloproteinase 7 (MMP-7) production. This was done was by an integrin-binding RGD (arginine–glycine–aspartic acid) motif via the extracellular signal-regulated kinase (ERK) pathway. It was shown that suppression of MMP-7 production by fibulin-5 resulted in inhibited metastasis in mice. Therefore, this ECM alteration in COPD and lung cancer cannot be involved in lung cancer development.

Fibronectin was observed to be increased in the ECM of both COPD and lung cancer. In general, fibrillar proteins such as fibronectins conver tensile strength to the lung[6]. Therefore, if levels of fibronectin increase, it can contribute to the increased stiffness of the ECM. As we have seen before, increased ECM stiffness can in turn upregulate integrin signalling[10] which can affect hallmarks of cancer such as proliferation, cell invasion and survival.

The last glycoprotein that has been observed to be altered in both lung pathologies is tenascin C. Tenascin C levels were found to be increased in the ECM. Both fibronectin and tenascin C induce MMP expression and activity[45]. It has been shown that MMPs and tissue inhibitors of metalloproteinases (TIMPs) levels are disrupted in COPD and lung cancer[46]. No studies have been found that report single MMPs that are consistently overexpressed in all tumor types. Tenascin C might induce all different kind of MMPs. Since every MMP has a slightly different function[46], activation of these MMPs attribute to cancer development in numerous ways. Therefore, fibronectin and tenascin C might indirectly influence tumor progression.

Another way in which fibronectin and tenascin C can contribute to lung cancer development is because they can modulate integrin-mediated adhesion of cells to other ECM proteins[33]. In this way, increased levels of fibronectin and tenascin C can contribute to cancer invasion.

Oskarsson T, et al. found that tenascin C enhances the expression of musashi homolog 1 (MSI1) and leucine rich repeat–containing G protein–coupled receptor 5 (LGR5), which are positive regulators of NOTCH signaling and target genes of the WNT pathway, respectively. It has been postulated that the NOTCH and WNT pathways support the fitness of initiating breast cancer during lung metastasis[47]. While this might be the case for breast cancer, it remains unclear if this also applies for lung cancer. Moreover, this process still does not explain how lung cancers originate. Oskarsson T, et al. also studied whether tenascin C had an effect on primary tumor growth, but this was not the case. So, there is yet no evidence that tenascin C can explain the increased risk to develop cancer for patients with COPD.

Another group of proteins in which differential expression was observed are the proteoglycans. Although reduced expression of proteoglycans in the alveoli of COPD patients has been observed, both in COPD and lung cancer there is a general upregulation of proteoglycans. Among the upregulated proteoglycans that is upregulates in both pathologies is hyaluronan (or hyaluronic acid). The function of hyaluronan is dependent on its molecular weight[48]. High molecular weight hyaluronan shows anti-angiogenic, anti-inflammatory and immune-suppressive effects. On the other hand, low molecular weight hyaluronan exhibits

pro-angiogenic and pro-inflammatory effecs[49, 50]. If hyaluronan accumulates, as is the case in COPD and in the microenvironment of lung tumors, there is also an accumulation of its fragments. These can activate the expression of inflammatory genes that in turn interact with immune cells to drive the immune response[7]. Hyaluronan can interact with signaling receptors such as CD44. This binding can lead to several processes. Firstly, it can promote cyclo-oxygenase-2 (COX2)-dependent invasion[51]. Secondly, it leads to the expression of MMP-2[52]. MMP-2 has been known to promotes secondary growth and cell sustainability in metastatic sites[53]. Thirdly, it is involved in survival of cancer cells, because it downregulates proapoptotic pathways[54] and lastly it prevents CD44-positive cancer cell death induced by cytotoxic T lymphocytes[54]. Therefore, in the latter two cases, hyaluronan can attribute to the increased risk to develop cancer.

To summarize, multiple similar alterations of the different constituents of the core matrisome have been found in COPD and lung cancer patients. While several of them were found to be involved in one or multiple hallmarks of the onset of cancer, others were only involved in processes that occur once the mature and/or metastatic tumor has already been formed. ECM alterations were also found that were not involved in cancer development, but conversely, in defense mechanisms to suppress cancer metastasis.

Discussion

A lot of the explanations that were given in this thesis on how ECM alterations might increase the risk to develop cancer, are suggestions. It must be considered with caution, because there is still a lack of actual experimental proof of what is happening in the tumor microenvironment.

There are more limitations in research focused on the link between ECM alterations of COPD that can increase the risk to develop lung cancer. While some findings quite clearly describe how changes of the ECM might increase the risk to develop cancer, some are vaguer. For example, it was found that increased tissue stiffness can increase integrin signaling and that this in turn can affect proliferation, cell invasion and survival. It is yet unclear which specific integrin signaling pathway is involved and which of the characteristics that cause the transition from a normal cell to a cancer cell is affected. The ECM is a highly dynamic structure in which its components are highly interconnected. Therefore, it is challenging to understand the molecular basis of remodeling processes. Aspects of cell behavior can include receptor signaling, rearrangements of the cytoskeleton, changes of gene expression that are all affecting each other[10]. Therefore, it is difficult to determine which specific change of the ECM is causal to the other and which ECM alteration lays at the basis for the other subsequent changes that are reactions to this initial change of the ECM.

In vitro experimental models, such as cell culture techniques, can help overcome this problem. With this technique, researchers can manipulate specific components of the ECM and observe the consequent responses. By specifically modifying single ECM components and studying subsequent alterations, researchers can increase understanding of causal interactions. Another way to specifically modifying single ECM components is by using 3D tissue engineering and organoid models. These models can mimic the native ECM environment. By manipulating the ECM composition, architecture, and mechanical properties it can give insight into causal molecular relationships. These techniques might be

interesting to study whether COL11A1 can indeed lead to exclusion of T-cells from the tumor microenvironment.

Although these techniques might be useful for initial research, cell cultures or 3D tissue engineering techniques cannot completely capture or comprise the compositional heterogeneity of the ECM nor the dimensionality of tissues[55]. To obtain a better understanding of the interconnectivity of ECM processes, there is a need for studies *in vivo*, such as the use of transgenic models. In this way, gene knockouts can be used or gene expression and signaling pathways can be adapted in mice. By studying the effects of these alterations, researchers can determine the impact on subsequent cellular responses.

An interesting study that could be performed is a study that identifies which specific MMPs are differentially expressed as a consequence of tenascin C and fibronectin upregulation in COPD. It has been found that tenascin C and fibronectin can induce MMP expression, but no studies have been found that report single MMPs that are consistently overexpressed in all tumor types. However, it might be possible that specific MMPs that are upregulated in COPD could contribute to to the subsequent onset of specific lung cancers.

Another study that could be performed is to study which specific integrin-mediated adhesion of ECM proteins to other cells is affected by tenascin C and fibronectin. This might give a better understanding of how these proteins influence cell invasion.

Lastly, it might be interesting to study if a specific molecular weight of hyaluronan is predominantly present in its accumulation in COPD and lung cancer. Since the function of hyaluronan is dependent on its molecular weight, its most predominant form in COPD might also influence the onset of lung cancer.

A lot of research has been done on mice. However, these studies also have their limitations. There are a lot of anatomical differences between the airways of the mouse and humans, such as lobar distribution, branching patterns, presence of bronchial submucosal glands, and goblet cells[56]. For this reason, conclusions that have been made in studies involving mice are more difficult to apply to humans. Simply, because anatomy can have an influence on observations that have been made.

There are also studies *in vivo* that have been performed including humans. However, there is a lack of large sample multicenter prospective cohort study data in clinical practice[22]. Moreover, most of the studies involving lung cancer have been focused on NSCLC. This is a logical choice, since 98% of lung cancers in COPD patients are NSCLCs[57], however, since lung cancer is the first cause of cancer mortality, other types of lung cancer still have a huge mortality. Therefore, it is important that other cancer types are studied as well.

Similar to the subtypes of cancer, studies on COPD also mainly focus on emphysema, rather than other sub-phenotypes that are often found in COPD[19]. Still, there are mixed conclusions regarding the relationship between lung cancer and emphysema[2]. Therefore, it is important that other sub-phenotypes are included in the studies as well.

To compare the ECM of patients with COPD and lung cancer, studies were found that focuses on either COPD or lung cancer[19]. While this is a good starting point, it would also be interesting to study patients that have both pathologies. Perhaps in such a research it appears that several ECM changes are more dominant than others in patient that have both pathologies. This might give an idea on which ECM alterations are more important in the onset of cancer.

To conclude, this thesis has given a number of mechanisms that could contribute the onset of cancer. If these mechanisms indeed are causal for the development of cancer, this would

explain why patients with COPD have an increased risk to develop cancer. Although there are some hypotheses on how ECM changes can increase the risk to develop lung cancer, more research in humans with bigger sample size and research that also includes patients with both pathologies are needed to confirm whether these hypotheses are indeed causal to cancer development.

Afterword

Writing this thesis has been a challenging journey. It was difficult to specify a topic, because I was not so familiar with the subject and I had not anticipated how much research had been done in this field. Therefore, it was sometimes hard to keep in mind what was most important to discuss and which findings were too specific to include in the thesis. My tendency is to dive too deep into a specific finding instead on focusing on the bigger picture. What I have learned during these four weeks is that it is important to stay critical on what you should focus on.

Before starting with the thesis, I joined a thesis ring group in which it was possible to share problems you encountered. After the first two sessions, I did not think it was of much help, because it occupied two hours of the week and it was also intended to give feedback on other students. Since I had some difficulties with writing my own thesis, my priorities were not so much on focusing on fellow student's work as well. It actually gave me more stress. However, during the third meeting I received very useful feedback on how I should make priorities. From that point on, I felt like my writing process became more effective. Overall, I think the writing process was more difficult than I anticipated, but I am glad with the result.

I would like to thank Dr doctor Corry-Anke Brandsma for wanting to supervise me on a very short notice. Also, I would like to thank her for helping me define the topic of the thesis, for her guidance, for her effort to take a critical look at my report and for the pleasant contact. Lastly, I would like to thank my second assessor as well for wanting to take a critical look at my report.

Rosalie,
July 10, 2023

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