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VASCULAR RELATED PATHOLOGIES IN CARDIOVASCULAR DISEASE AND CANCER

PATOLOGIA NACZYŃ KRWIONOŚNYCH W CHOROBACH UKŁADU KRĄŻENIA I CHOROBACH NOWOTWOROWYCH

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Summary

Cancer and Cardiovascular diseases (CVD) are the two most prominent causes of death worldwide. Emerging evidence indicates shared risk factors and a common biology between these diseases. For instance, chronic inflammation has a significant role in contributing to both diseases. An alteration of the vasculature and the endothelial cells plays a key role in pathogenesis of CVD and cancer. The widespread overlap regarding disease prevention and risk factors for these diseases suggest a common mechanism in terms of molecular pathways. The goal of this tutorial is to present common problems and mechanism of these two mayor diseases.

Keywords: cancer, vascular, mechanism, diseases

Streszczenie

Choroby nowotworowe i sercowo-naczyniowe (CVD) to dwie najczęstsze przyczyny śmierci na całym świecie. Pojawiające się dowody wskazują na wspólne czynniki ryzyka i wspólną biologię między tymi chorobami. Na przykład przewlekły stan zapalny ma znaczącą rolę w przyczynianiu się do obu chorób. Zmiana układu naczyniowego i komórek śródbłonka odgrywa kluczową rolę w patogenezie CVD i raka. Czynniki ryzyka tych chorób sugerują wspólny mechanizm pod względem szlaków molekularnych. Celem tego artykułu jest przedstawienie typowych problemów i mechanizmów tych dwóch chorób.

Słowa kluczowe: rak, miażdżyca, śmiertelność, mechanizmy

1. General Introduction

Cancer and Cardiovascular diseases are the two most prominent causes of death worldwide [1]. Emerging evidence indicates shared risk factors and a common biology between these diseases. For instance, chronic inflammation has a significant role in contributing to both diseases [2, 3]. An alteration of the vasculature and the endothelial cells plays a key role in pathogenesis of CVD and cancer [4]. The widespread overlap regarding disease prevention and risk factors for these diseases suggest a common mechanism in terms of molecular pathways [5].

1.1.1. Vascular Ageing

A key factor that contributes to vascular dysfunction is ageing reliant injury of normal endothelial functioning. This is responsible for numerous age-related diseases of the vascular system and other organs. All organs undergo a progressive decline of function and structure over time due to ageing. Endothelial cells undergo senescence during this process and display substantial changes in their properties ensuing damage to the vascular functionality and neo-angiogenic capability. Thus, changes to mechanical and structural properties of the vascular wall result in damage of arterial elasticity and reduced arterial compliance [6, 7].

Evidence suggests that different disease state like diabetes, hypertension and end stage renal failure show a reduced arterial compliance. These changes could also be present before the manifestation of CVD. Vascular ageing contributes to the ageing dependent growth in atherosclerotic disease and hypertension. More than traditional risk factors like lipid levels, smoking, diabetes, and sedentary lifestyle, ageing is a factor that deliberates a greater risk for the disease. Mitochondrial dysfunction, microRNAs and micro environmental

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stressors like hypoxia, Mechanisms like mitochondrial dysfunction and micro environmental stressors, are defined to be involved in ageing-related endothelial cell senescence control [8, 9].

The process of ageing is characterised by a functional decline in the cells and tissues and the diminished ability to suitably respond to environmental distress, including metabolic stress and reduced oxygen supply. This results in deterioration of the total fitness of the organism, which is often associated with the individual's life style [10-12]. As the vasculature is the major source of oxygen and nutrient supply in the body, endothelial cells are extremely susceptible to deviation in oxygen pressure. The age-related injury of the response to oxygen and nutrients level variation is considered a key factor contributing to arterial dysfunction that leads to age related vascular diseases, for example, atherosclerosis [13-16].

1.1. Cardiovascular Disease

Cardiovascular diseases are a set of diseases involving the heart or the blood vessels [17, 18] and are the foremost cause of death and disability in the world. CVDs include, coronary artery diseases such as myocardial infarction and angina, congenital heart disease, heart arrhythmia, heart failure, stroke and peripheral heart disease to name a few. The underlying mechanisms vary depending on the disease. For example, coronary artery disease, peripheral artery disease and stroke involve atherosclerosis [15, 16, 19, 20].

1.1.1. Causes

1.1.1.1. General Mechanism

Many years ago, atherosclerosis was thought to be a lipid storage disease. Lipid deposits were moulded on artery's surfaces and grew until they became large enough to hinder blood flow and eventually result in a cardiac event such as a myocardial infarction or a stroke [3]. Current mechanisms involve inflammation, which play a key role in atherosclerosis formation, right from its initiation and development to its endpoint-thrombotic complications. [21] ECs, which form the innermost layer of the arterial wall, normally resist the attachment of leukocytes such as macrophages and T lymphocytes from binding to its wall. But triggers like consuming high saturated fat diet, hypertension or smoking can initiate this binding. One factor is vascular endothelial growth factor-1 (VCAM-1) [22].

Lesions also develop due to type of blood low they experience. Shear stress occurs due to laminar blood flow and this result in several atheroprotective mechanisms [22, 23]. For example, it produces an antioxidant enzyme, superoxide dismutase or an increased expression of nitric oxide synthase. This limit VCAM-1 expression by inhibiting nuclear factor kappa beta(nF-Kbeta) production and thus combat platelet clumping [24].

Once adhered, the monocytes penetrate the endothelium and infiltrate the intima by diapedesis a process that requires a chemoattractant gradient such as monocyte chemoattractant protein -1(MCP-1) [25]. Within the intima, monocytes change into macrophages, and express scavenger receptors and engulf lipid particles, thus transforming into foam cells characteristic of atherosclerotic lesions.[26-28]. In the intima through lesion evolution the T lymphocytes join macrophages and secrete cytokines and growth factors that may nurture the migration and proliferation of smooth muscle cells [29-32]. T lymphocytes also excite macrophages to produce collagen-degrading enzymes and secrete cytokines and growth factors that may promote the migration and proliferation of smooth muscle cells. This leads to the fibrous cap that protects the blood from the thrombogenic core of the plaque, to weaken. This results in rupturing of the plaque leading to thrombosis which is the complication in most atherosclerotic cases [31, 33, 34].

1.1.1.2. Dysfunctional endothelium

A normal vasculature has an organised network of blood vessels maintained by a balance between pro and anti-angiogenic factors. A healthy tissue also displays a systematic network of lymphatic vessels that allows for transport and draining of blood and metabolic waste from the interstitium. This intricate architecture consisting of mature vessels make up the normal vasculature. These vessels allow the adequate transport of nutrients, oxygen and blood supply required to sustain the vasculature [35, 36] An alteration to the vasculature plays a significant role in the pathogenesis of various diseases.

The endothelium plays a significant role in governing the circulation as a physical barrier and as a variety of different regulatory substances. For example, the endothelium derived prostacyclin and nitric oxide inhibit platelet function and induce vascular relaxation when they are released in response to physical stimuli, platelet derived substances or hormones. The endothelium is also a good source of heparins, heparin

sulphates, thrombospondins and platelet derived growth factor. Also, several vasoactive substances produced by the endothelium such as nitric oxide, angiotensin- 2 and endothelin-1 might play a role in vascular tension. Dysfunction of these endothelium dependent factors could lead to CVD such as atherosclerosis and hypertension and is thought to be involved in stroke, tumour angiogenesis, vascular leakage and infectious diseases [37, 38].

Endothelial dysfunction occurs when the endothelium shifts to a pro inflammatory, reduced vasodilatory and pro thrombotic state. This state is associated with most forms of cardiovascular diseases. When free radicals disrupt the balance of NO in the body, damage to the endothelium occurs leaving them excessively permeable allowing for toxins to pass into the tissues.[39] When the action of NO is inhibited, endothelial signalling is weakened resulting in widespread disease, since in the human body, the endothelium actively maintains, around 66,000 miles of blood vessels. A normally functioning endothelium also supports the body's immune response, helps regulate blood clotting, controls the volume of fluid and the number of electrolytes and other substances passing from blood into the tissues, and produces dilation or constriction of the blood vessels.

1.1.1.3. Hemodynamics

The dynamics of blood flow is called hemodynamics. Blood, being a non-Newtonian fluid, is best studied with rheology [40].

Normal blood flow ensures the transportation of nutrients, oxygen, CO2, metabolic waste throughout the body thus enabling regulation of different functions such as maintaining cell metabolism, pH and osmotic pressure and temperature regulation along with protecting the body from harmful stimuli. These are key factors that help the body adapt as per the environment. A laminar blood flow thus ensures proper functioning of the body while a turbulent flow and local hemodynamic factors like flow disturbances at bends and bifurcations contribute to the formation of atherosclerosis or other pathological conditions. A laminar flow occurs when the vessel wall is smooth whilst a turbulent flow occurs when there is a decrease in wall smoothness. This is due to fatty deposits on the vessel wall.

The vascular ECs form the innermost layer of the vessel wall with direct contact to blood flow and are involved with vital homeostatic functions to various mechanical and chemical stimuli. Not only do they provide a selective barrier for permeability but the ECs also influence hemostasis and thrombosis through secretion of pro and anti-coagulants, fibrinolytic agents and mediate inflammatory responses via the release of cytokines and chemokines. The EC's also regulate smooth muscle migration through the release of vasodilators and constrictors and influence vascular remodelling using growth promoters and inhibitors. Hence hemodynamic forces are needed for normal physiological functioning of the ECs while some forces induce a dysfunction of the endothelium by modulating EC gene expression and signalling leading to the development of pathological states which contribute to the formation of atherosclerosis, thrombosis and its complications.

The role of hemodynamic forces in endothelial dysfunction was first proposed when observations of initial atherosclerotic lesions were credited to a nonrandom pattern of development. These were observed typically at arterial bends and branches with a disrupted flow. This flow pattern included recirculation eddies and change in direction with respect to space (reattachment and flow separation) and time (reciprocating flow).

Recent research show that this kind of flow and the associated reciprocating and low shear stress bring about a constant activation of several atherogenic genes in ECs. for instance, the monocyte chemoattractant protein (MCP-1) that induces monocyte infiltration into arterial wall, and platelet derived growth factor (PDGF) that augment EC turnover and SMC migration, into the subintimal space [28].

On the contrary, the straight portion of the artery, usually safe from the atherosclerotic lesions, is exposed to constant laminar blood flow and high shear stress, all with a definite direction of flow and the associated downregulation of atherogenic genes and upregulation of growth arrest genes and anti-oxidants in EC's. Hence these findings propose that laminar and turbulent flow patterns might induce a variety of molecular responses in ECs which result in sparing of the straight parts of the arteries and formation of lesions at the curvatures.

1.2. Blood and Blood Vessels

1.2.1. Blood

Normal tissue functioning requires an adequate supply of oxygen, nutrients and blood vessels to facilitate their transport. Virchow, around a hundred and fifty years ago, explained vascular diseases in terms of cellular mechanisms, most of which stand valid till date ®. However, more recently the vessel wall has been visualized as a channel containing blood, which is provided by the heart pumping around blood in a circuit that is optimized to exchange and distribute oxygen and nutrients.

Blood is a body fluid that delivers oxygen and the essential nutrients to cells and tissues [41]. In vertebrates, blood is comprised of blood cells suspended in blood plasma. Blood has various functions such as, supplying oxygen to different tissues, supply of nutrients like amino acids, fatty acids and glucose, waste removal (CO2, urea) etc. Blood cells constitute the following components; erythrocytes, leukocytes, platelets. The most abundant are the erythrocytes, which has the iron containing protein, the hemoglobin which facilitates transport of oxygen [42-44].

The cellular components of blood are formed through a process known as hematopoiesis, which is a process that occurs during embryonic development and throughout adulthood. All the cells are derived from the hematopoietic stem cells. In a healthy adult, around 10^11-10^12 new cells are formed every day. The process occurs in two waves. The primitive wave consists of an erythroid progenitor which appear in blood islands, giving rise to macrophages and erythrocytes during initial stages of embryonic development. The main purpose of this stage is to provide tissue oxygenation since the embryo grows rapidly. This wave is transitionary. In humans, foetal haematopoiesis begins in the yolk sac and shifts to the liver temporarily before establishing in the bone marrow and thymus when the baby is born.

Bone marrow is the tissue encompassing the centre and the epiphysis of bones. It is also the place for production and maturation of B cells. It is an organ composed of trabecular and cortical bone, cartilage, hemopoetic and connective tissues. The trabecular bone is composed of a framework of fine bone plates filled with hematopoietic marrow, fat containing marrow or blood vessels. The bone marrow consists a vascular component (stroma) and a hematopoietic component (parenchyma) The parenchyma includes hematopoietic stem cells (HSCs) and hematopoietic progenitor cells, localized near the endosteum of the bone and more around blood vessels. The stroma comprises multipotential non-hematopoietic progenitor cells as well which can differentiate into various tissues of mesenchymal origin, including, endothelial cells, osteoblasts, reticular cells, adipocytes and fibroblasts. The stromal cells including ECs deliver signals for migration of specific leukocytes into and out of the bone marrow, involving in rolling/extravasations along the vascular endothelium [45-48].

1.2.2. Blood Vessels

Angiogenesis is a process through which fresh blood vessels are formed from existing vessels. It is a normal and key process that occurs throughout life and is involved in development and growth, disease as well as wound healing. Oxygen plays a critica l role in this process along with hemodynamic factors that are essential for the survival of vascular networks and for structural adaptations of vessel walls.

The blood vessels originate through a process beginning with the mesodermal layer. The first system to develop in the embryo is the cardiovascular system. The luminal surface of this system that is in close contact to the blood is made up of a single layer of endothelial cells derived from the mesoderm. It is formed by the following processes. Hematopoietic stem cells and angioblasts arise from the differentiation of hemangioblasts followed by vasculogenesis which is the de novo formation of blood cells from angioblasts. It is an active process that involves cell to cell interactions along with cell to extracellular matrix interactions. This process is directed spatially and temporally by morphogens and growth factors. Vasculogenesis includes differentiation of mesoderm stem cells into angioblasts followed by migration of angioblasts to form blood islands where they give rise to endothelial cells [45-48].

There are two types, the sprouting and intussusceptive angiogenesis. Sprouting angiogenesis occurs when sprouts of endothelial cells migrate towards a stimulus, in this case VEGF-A. It thus adds vessels to parts of tissues that previously lacked blood supply. Intussusceptive, on the other hand forms blood vessels by splitting vessels due to which interstitial fluids invade an existing blood vessel [49, 50].

The steps involved in sprouting angiogenesis process are: Basement membrane degradation followed by endothelial cell (EC) proliferation, focused migration of the cells, formation of new vessels (tubulogenesis), fusion of the vessels, vessel pruning and pericyte stabilization. The process begins in response to a hypoxic environment. VEGF-A initiates the process. An endothelial tip cell responds to this stimulant by guiding the sprouting EC through the ECM towards VEGF-A. The ECs migrate through the ECM via filopodia, which are long, thin projections which extend from the migrating tip of the cells. These secrete proteolytic enzymes which degrade the basement membrane. When a sufficient number of filopodia on have anchored to the substratum, the tip cell is aligned with the VEGF-A receptor through contractions of actin filaments within the tip cell. Meanwhile, the capillary sprout elongates due to endothelial stalk cell proliferation, since they follow the tip cell causing the elongation process. A lumen is formed within a series of stalk cells due to vacuoles developing and coalescing. These stalk cells form the trunk of the recently formed capillary. When the tip cells of two or more capillary sprouts meet at the source of VEGF-A secretion, the tip cells merge together forming a continuous lumen via which oxygenated blood can flow. VEGF-A levels return to near normal, when the local tissues receive suitable amounts of oxygen. Pericyte recruitment induces maturation and stabilization of the capillary [7, 31, 32, 48].

Intussusceptive angiogenesis also called splitting angiogenesis, is a process where a single vessel splits in two due to an extension of the vessel wall into the lumen. This type is considered to be quick and effective compared to sprouting angiogenesis since, initially, it only requires restructuring of existing ECs and does not require immediate EC proliferation or migration. Intussusceptive angiogenesis occurs lifelong but plays an important role in vascular development in embryos where growth is quick and resources are limited. Intussusception mainly causes new capillaries to develop from existing capillaries.

All blood vessels except for capillaries are made up of three layers, tunica intima, tunica media and the adventitia. The tunica intima, the thinnest layer is comprised of a monolayer of endothelium cells that line the vessel wall, surrounded by an elastic connective tissue called the internal elastic lamina, whose function is to related to elastic resilience to sustain blood pressure [34, 51-53]. The tunica media is made up of numerous layers of elastic lamina and smooth muscle cells. This is the thickest layer. The SMC's present in the medial layer influence blood pressure through the production of multiple ECM components (elastin, collagen) [54]. The adventitia is comprised of loose connective tissues made up, primarily of collagens and elastic fibres, fibroblasts and macrophages. The fibroblasts play an important role in fibrognesis. The adventitia also plays a significant role in vascular remodelling and development of vascular diseases such as transplant vasculopathy, atherosclerosis, hypertension and restenosis [55, 56].

1.3. Vascular Remodelling

Vascular remodelling is an intricate process of structural changes that involve at least four different process – cell growth and death, cell migration and degradation of extra cellular matrix (ECM). The process is dependent on an interplay between locally generated growth factors, hemodynamic stimuli and vasoactive substances.

Generally remodelling is an adaptive process which occurs in response to long term changes in the hemodynamic conditions, but it might eventually contribute to pathophysiology of circulatory disorders and vascular diseases [57, 58]. For instance, physiologically, vascular remodelling occurs during pregnancy. Adequate uteroplacental blood flow is needed for a normal pregnancy outcome. This process takes place with the growth and remodelling of the uterine circulatory system along with growth of a new organ, the placenta.

Vascular remodelling involves various patterns and the nomenclature used to denote these patterns is circumferential remodelling. It is normally called inward or outward to signify narrowing vs. widening of the vessel wall. The term expansive remodelling is used to denote increase in circumference and it is employed as a substitute for outward Also, taken into consideration is the wall mass, which can increase (hypertrophy), decrease (hypotrophy) or remains unchanged (eutrophy). For example, the remodelling of vessel to larger lumen with the same wall thickness occurs during pregnancy in the uterine circulation. This is called outward hypertrophic because the cross-sectional area is increased. This is due to the diameter of the chief uterine artery almost doubling in magnitude in humans during pregnancy and the enlargement occurs with or without the thickening of the vascular wall. Another study on human myometrial radial arteries from preeclamptic women the pattern of remodelling was inward eutrophic since there was no change in cross-sectional area indicating a rearrangement of existing wall elements around a smaller lumen.

Several factors contribute to vascular remodelling resulting in pathological implications. For example, when pulsatile blood flow and pressure induce various kinds of hemodynamic forces such as shear stress, hydrostatic pressure, constantly on blood vessels. Since the ECs are in direct contact with the blood, they bear most of the shear stress as a result of frictional forces arising from blood flow acting parallel to the vessel luminal surface. This increase or decrease in shear stress plays a key role in vascular remodelling and homeostasis. During the process of remodelling, for a compensatory arterial response towards changes to occur, a functional and intact endothelium is required to undergo adjustments in function and structure in response to alterations in shear stress.

Vasoactive factors also play an important role in vascular structure determination. They have an acute effect on vascular muscle tone and they may also impact matrix production and migration of cells. Angiotensin 2, for example, induces growth of vascular smooth muscles cells through PDGF AA and TGF beta 1. They are also involved in activation of EC through receptors coupled to ion channels which in turn modulate intracellular calcium concentration.

Vascular remodelling can also occur in response to increased arterial pressure, in which case, the structure of the vessel wall is modified such that the ratio of the width of the wall to the width of the lumen is raised either, by an increase in muscle mass or rearrangement of cellular and non-cellular elements. This results in heightened peripheral resistance typical for hypertension [59-61].

Another form of remodelling includes reduction in lumen diameter and is related to other diseases such as cardiac allograft vasculopathy and restenosis post percutaneous intervention [62]. Alternatively, remodelling

could also lead to higher lumen diameter and compensate for increased plaque load [63]. Vascular remodelling also involves changes primarily in lumen dimensions due to active reorganization of wall components. This type is associated with constant high blood flow, observed in patients -an arteriovenous fistula and was shown in animal models [64, 65].

Remodelling of vessel wall also occurs in response to vascular injury. A neointima forms in a sequential process as part of a reparative answer to injury. This involves thrombosis, migration and proliferation of vascular cells, matrix production, and inflammatory-cell infiltration. Mechanical injury results in vessel constriction and smaller vascular lumen in response to the scarring in the outer vessel layer [66]. Key features of transplant vasculopathy include inflammation and formation of intimal hyperplasia [62, 67, 68].

1.3.1. Vascular injury

Vascular trauma is the injury caused to a blood vessel- the artery, that carries blood to an organ, vein, which returns blood back to the heart.[69] Hemodynamics, hypoxia, ischemia and endothelial dysfunction are factors that contribute to vascular injury. Vascular injury is brought about by mechanical injury due to surgical manipulations and from tissue ischemia due to obstruction of the vasa vasorum. The level of the resulting neointimal lesion is related to the extent of vascular injury.

Distressed flow patterns contribute to pathogenesis of various clinical disorders such as atherosclerosis, arterial aneurysm, post-surgical intimal hyperplasia, ischemic/reperfusion injury. This is due to disturbances of blood flow in arteries generated due to surgical interventions like end to end anastomosis in bypass graft or stent insertion in balloon angioplasty. Additional flow disturbances that facilitate endothelial dysfunction is the termination or inactive flow and its recovery later in clinical conditions related with ischemia/reperfusion and hypoxia injury. Hence myocardial recovery following acute infarction becomes complex and the outcome could be cell damage, arrhythmia and death. Tissue injuries induced by solid organ transplantation, tissue resuscitation and key vascular surgical intrusions could also result in a dysfunctional endothelium. This results in inflammatory responses with recruitment of WBCs from circulation.

Another mode of injury occurs through ischemia/hypoxia. Hypoxia is a vital component of an ischemic result. Hypoxia is the reduction of oxygen while ischemia is the lack of perfusion. Ischemia is a process which occurs when the tissue's demand for energy substrates is not met with supply. Reperfusion injury or ischemic/reperfusion injury occurs when blood flow and oxygen resume to a tissue after a hypoxic/ischemic event. HIF 1 alpha is stabilized in response to limited oxygen. This is worsened by modification of locally released vasoactive mediator such as NO and its action. Sudden re-oxygenation post hypoxia activates the release of free radicals, like reactive oxygen species (ROS) and reactive nitrogen species(RNS), which modify EC homeostasis and cause swelling and tapering of the blood vessel lumen along with a host of other damaging effects. ROS and RNS are highly reactive molecules and include hydrogen peroxide, peroxynitrite, hydroxyl radical, and superoxide resulting in oxidative stress, disparity between the production of reactive species and antioxidant defences that causes tissue damage. Tissue injury is characterized by a loss of tight junctions, leading to augmented permeability, detachment from the basement membrane, and, often, EC apoptosis or necrosis. Eventually all of these structural changes contribute towards microvascular perfusion impairment.

1.3.2. Intimal hyperplasia

Intimal hyperplasia is the universal response of a vessel to an injury. It is the thickening of the tunica intima [70]. It is connected to increased cell number and the amount of ECM in the intimal layer of the vessel[71]. Physiologically, it occurs in the involution of the uterus, during closure of the ductal arteriosus post birth (DA) In the foetus, the process of intimal thickening starts in the second trimester of pregnancy with the build-up of glycosaminoglycans in the sub endothelial region (SER). This is followed by separation of ECs from the internal elastic lamina, followed by migration of SMCs into the sub endothelial region. This phenomenon was also observed in the mature DA in the neonate, indicating that this is a constant process [71].

Pathologically, it occurs post balloon angioplasty, transplantation, artery bypass conduits, in pulmonary hypertension and pre-atherosclerotic lesions [15, 16, 34, 51, 72].

Intimal hyperplasia occurs in a few stages. The key stimuli are inflammation, injury and enhanced vessel wall stress. For instance, balloon injury to the rat carotid artery does not incite a marked inflammatory response but nonetheless generates intimal hyperplasia. Inflammation is, nevertheless, a confounding feature of most other models of vascular injury and therefore may contribute to the extent of intimal hyperplasia. Direct effects of inflammatory mediators on early transduction events, mainly the NF-κB pathway, have been implicated. Synergistic communication between growth factors and inflammatory cytokines to cause MMP induction and

activation is another probable mechanism. In addition, proteases and growth factors directly derived from macrophages possibly play key roles.

Two situations which evidently demonstrate a relationship between increased mean wall stress and intimal hyperplasia are vein grafting and pulmonary hypertension. The mediators involved remain largely undefined but increased MMP and PDGF expression has been observed in experimental vein grafts and was reversed in parallel with intimal hyperplasia when the grafts were supported by an external stent.

The injured artery recruits inflammatory cells such as macrophages and leukocytes and mobilizes vascular progenitor cells from their niches. Another important factor in the neointima formation, platelet-derived growth factor (PDGF), FGF along with additional factors, is produced by platelets, smooth muscle cells, endothelial cells and macrophage foam cells. These factors along with PDGF act as chemo attractants which promote cell migration of the SMC's into the neointima from the media. Also, PDGF promotes production of collagens and proteoglycans. In parallel, the ECM is remodelled by MMP's which further promote SMC migration. Thus, the neointima grows in response to cell proliferation, increased ECM synthesis, apoptosis and fibrosis [73-75].

Various sources of cells contribute to neointima formation. They can originate from the adventitia from cells such as pericytes, fibroblasts and vascular progenitor cells or from circulating progenitor cells, for example-endothelial progenitor cells, smooth muscle progenitor cells or bone marrow derived cells.

1.4. Cancer

Cancer is a disease involving abnormal cell growth, which arises when cells undergo uncontrolled proliferation and lose their ability to control apoptosis. More than two hundred diverse types of cancer exist, and their etiology remain uncertain. Current hypothesis state that cancer is a genetic disease and numerous mutations in various genes are considered as cancer promoting genes. Additional factors such as lifestyle, obesity, tobacco, alcohol consumption and virus infections constitute risk factors for cancer development [72, 76-79].

Dysfunctional endothelium is a hallmark of many diseases like diabetes mellitus, atherosclerosis and cancer. Endothelial cell migration is an essential component of angiogenesis and requires a tight regulation of the contractile and noncontractile conditions of the cell. These processes require the combination of signals elicited by hepatotactic, chemotactic and mechanotactic stimuli. This movement is in turn, related to the activation of intracellular pathways that congregate on cytoskeleton remodelling.

There are several types of malignancies that affect humans and they are classified based on the type of cell that the tumour cells resemble. These include carcinomas representing a group of cancers originating from epithelial cells, including the most common cancers breast, colon, lung and prostate cancer, - Sarcomas a neoplasm derived from connective tissue (e.g. bone, cartilage, fat), originating from mesenchymal cells outside the bone marrow and lymphomas and leukaemia's: neoplasm arising from blood cells that leave the bone marrow and mature in lymph nodes and blood.

1.4.1. Normal and tumour vasculature

There are fundamental differences between the normal and tumour vasculature. The normal vasculature has an organized network of blood vessels maintained by a balance between pro and anti-angiogenic factors. A healthy tissue displays a systematic network of lymphatic vessels that allows for transport and draining of blood and metabolic waste from the interstitium. This intricate architecture consisting of mature vessels make up the normal vasculature. These vessels allow the adequate transport of nutrients, oxygen and blood supply required to sustain the vasculature [36, 80].

The tumour microenvironment consists of different cell types such as endothelial cells, pericytes, and fibroblasts. These cells contribute through the rearrangement of the ECM and secretion of various growth factors and cytokines to tumour progression.

In tumours, aggressive neoplastic growth is accompanied by an over expression of pro angiogenic factors which lead to the formation of aberrant blood vessels. These blood vessels are immature and highly permeable and give rise to a disorganized vascular structure comprised of irregular vessels with different shapes and diameters. This is due to the scarcity in smooth muscle cells and possible discontinuous endothelial cell lining with an abnormal basement membrane. Augmented vessel permeability leads to deviation in osmotic forces that results in build-up of vascular contents and high interstitial fluid. Blood flow is hindered due to resistance caused by the defective shape of the blood vessels. This, in turn, leads to insufficient oxygen supply with a localized hypoxia.

Other aberrant characteristics of the tumour vasculature include arteolar-venous shunts abnormal bulges and plasma channels lacking red blood cells. The typical blood vessel arrangement found in the healthy tissue (consisting of arterioles, arteries and venules) sometimes cannot be identified. The vessel endothelial cells are dysfunctional

and loose their expression of endothelial markers. The lymphatic vessels in tumours are also leaky, unstable and dilated. Due to this type of arrangement, various functional processes within the tumours are drastically different to those of the healthy tissue. For instance, the normal processes of nutrient delivery through these dysfunctional blood vessels and of metabolic waste removal via the lymphatic system are greatly reduced [36, 81].

1.4.2. Angiogenesis in cancer

Angiogenesis is a process through which new blood vessels form from existing vessels. It is a normal and key process involved in development and growth, as well as in wound healing. It is however, also involved in cancer, since it is the fundamental step for a tumour to grow in size and metastasize. Tumour angiogenesis was first reported in 1971 by Judah Folkman. He suggested that tumour growth is dependent on angiogenesis [82-85].

For a tumour to grow (more than 1-2 mm in diameter), it needs an independent blood supply, hence angiogenesis is a necessary step. The tumour secrets growth factors that recruit new blood vessels. The process continues after a tumour matures and is a vital step in sustained tumour growth and for tumour metastasis [86-88].

The newly formed vessels provide an exit route for tumours cells, through which cells may detach from the primary tumour and enter the blood stream. The angiogenesis process is regulated by the production of various angiogenic stimulators. This includes members of the VEGF and FGF families along with regulating angiogenic inhibiting factors like angiostatin and endostatin. The latter regulate and modulate the process both at the primary and metastatic sites. Vascular density plays an important role as a prognostic factor for many tumours. Highly vascular tumours are more metastatic [89, 90].

Regulation of angiogenesis occurs through hypoxia or ischemia. Many proangiogenic factors and their receptors maybe modulated by this process. For example, factors like VEGF, FGF, and TGF-beta, PIGF and angiopoietins and HIF. Oxygen deficiency stimulates and regulates HIF which in turn triggers genes for VEGF-A and VEGFR 1. The VEGF family is the most important player in angiogenesis [91-97].

VEGF induces vascular endothelial cell proliferation and growth and survival. The VEGF family consists of various receptors, namely VEGF- A, VEGF-B, VEGF-C and VEGF-D with their receptors VEGF -1, VEGF-2 and VEGF-3. VEGF-A along with the receptor VEGF -2 are important regulators of angiogenesis.

VEGF initiates angiogenesis by binding to specific receptors. When a tumour needs to grow, it releases growth factors, VEGF which binds to the extracellular receptor on the endothelial cell on the blood vessel. VEGF A, B and PIGF bind to VEGF 1, while VEGF A, C and D to VEGF -2. VEGF C and D bind to VEGF 3 on the endothelial cell of the lymph vessel thereby stimulating lymph angiogenesis [87, 98].

Once VEGF binds, dimerization of the receptors takes place which activates intracellular tyrosine kinase domain (ITK) thereby inducing auto phosphorylation. This further activates downstream signals required for the angiogenesis process [73-75, 99].

1.4.3. Colon cancer

Colon cancer is one of the foremost causes of cancer-related deaths worldwide. The chief cause of mortality in colon cancer patients is liver metastasis, either present already at cancer diagnosis stage, or developing after primary tumour resection. Survival rates of patients continue to increase with time, mainly because of improved diagnostics and treatment. Colonic epithelium consists of roughly ten million invaginations, called crypts whose base of contain multiplying daughter cells and dividing stem cells and form the starting point for the cell migration towards the epithelium surface. Here the cells die and become replaced by continuously streaming new cells.

One of the factors or an amalgamation of chromosomal instability(CIN), CpG island methylator (CIMP) phenotype, microsatellite instability(MSI) contributes to colon cancer progression. Genetic variability is usually caused by loss of heterozygosity and aneuploidy. Alternatively, mutations in the cell cycle genes or tumour suppressor gene may also lead to cellular transformation. Similarly, microsatellite instability and mutator phenotype are caused due to epigenetic and/or genetic modifications resulting in impaired cellular pathways, such as DNA repair mechanism. Non-coding RNAs, more prominently microRNAs and long non-coding RNAs have also been implicated at numerous CRC stages.

The acquisition of mutations in the adenomatous polyposis coli (*APC*), followed by the mutational activation of oncogene *KRAS* and the inactivation of the tumour suppressor gene, *TP53* signals the beginning of the CIN pathway. The major players in CIN tumours are loss of heterozygosity (LOH) and aneuploidy. This not only constitutes most of the sporadic tumours (85%) but also involves familial adenomatous polyposis cases

linked with germline mutations in the *APC* gene. Promoter hypermethylation of several tumour suppressor genes is a characteristic feature of CIMP pathway most importantly *MGMT* and *MLH1*. This is often associated with *BRAF* mutation and microsatellite instability. Inactivation of genetic alterations in short repeated sequences contributes to the MSI pathway. Also, hypermethylation of DNA mismatch repair gene might lead to MSI.

Both genetic and environmental factors contribute to histopathological changes. Key environmental factors involve toxins, pathogen invasion, polyamines, ROS (reactive oxygen species) production and stress. The growth of adenomas is triggered by adverse conditions like bacterial or viral invasions, subsequently causing mutation in the APC (Adenomatous Polyposis Coli) regulatory pathway usually affecting either APC or β-catenin. APC represses β-catenin, which diminishes the tendency to abnormal tissue expansion by augmenting protein expressions that promote and affect cell division and cell adhesion. As cells migrate from base crypts towards the epithelium surface, APC expression increases and hinders β-catenin. This in turn promotes apoptosis at the surface and provides optimal balance in production from the crypt base in parallel.

Transformation of dysplastic epithelium (stage I) to early adenoma phase (stage II) takes place due to COX2 mutations and appear in most human colorectal adenocarcinomas. This is followed by mutations in RAS genes, with K-RAS being the most common gene and less H-RAS the least. K-RAS mutations, next to common mutations in DCC, MLH1 and MSH2 facilitate the transition from early to late adenoma (stage III). Lastly, progression to tumour metastasis (stage IV), involves genes such as BAX, E2F4, MSH3, MSH6, TGF-βR2, BAX and MMPs, p53 and SMAD4 affecting liver, lungs, bone and brain.

1.4.4. Metastasis

Metastasis is a multi-step process involving a modulation of phenotype of cancer cells, invasion of cells to enter circulation and form distal metastasis. It is one amongst the three hallmarks of malignancy with circulating tumour cells being the prime cause in the formation of distal metastasis.

Metastatic disease accounts for majority of cancer related deaths. Metastases are believed to develop from dormant circulating tumour cells that are seeded into various organs. The process of metastasis formation involves an invasion-metastasis cascade of events. A stepwise progression is proposed. First, the normal cells undergo genetic alterations leading to the formation of pre-malignant lesions [100]. These steps are clinically recognized. Several types of pre-malignant lesions such as hyperplasia or dysplasia, can be detected in different organs prior to the formation of a malignant tumour. The lesions are caused either by genetic alterations or by an external factor (for example, a virus infection); the former causes a monoclonal expansion of cells, whereas the latter causes a polyclonal expansion of cells. Unknown factors precipitate pre-malignant lesions to develop into cancer. Further progression leads to invasive cancer, with a substantial risk of metastases.

A metastatic tumour is composed of cells that are phenotypically different and heterogeneous, when compared to a noninvasive tumour. They are believed to origin form circulating tumour cells that have left the primary tumour and can reach distal organs through the lymphatic system or via the blood circulation. Once there, they extravasate and invade the parenchyma and form micro -metastasis that can later develop into macroscopic metastasis. Loss of endothelial cell integrity and a selective permeability of the endothelium provides for the transmigration process [101-106] and several factors including an inflammatory environment are believed to aid in the survival of tumour cells in other organs.

Angiogenesis also provides an exit route for metastasis. This is due to the nature of permeable and immature vessels formed within the tumour leading to cells detaching and entering circulation. Thus, a highly vascular tumour gives rise to more metastasis compared to a less vascularised tumour. Butler. TP showed that about 2x10^6 mammary carcinoma cells are shed from the primary tumour each day, giving rise to metastasis [107].

The most important correlation between angiogenesis and metastasis are the studies on vascular density of the tumour and patient survival. A study by Weinder et al showed a direct a link between tumour vascularisation and metastasis, and indicated that it can function as an independent prognostic factor for outcome. This study was repeated by others and the findings were confirmed and the study is not limited to breast cancer. Thus, these studies show the importance of vascular density [104, 108].

The blood system is considered as the main mode for metastatic spread, but increasing evidence shows that the lymphatic system could play an important role in metastasis [109].

Lymphangiogenesis is the process of formation of new lymphatic vessels from preexisting vessels. It plays an important role in homeostasis. An impaired vessel or excessive formation of the vessel leads to metastasis. Generally, lymphatic vessels were thought to be indirect participants in tumour metastasis They provide conduits for tumour cells to transit into draining lymph nodes, but recently the discovery of several key lymphatic-specific molecular markers and an increased accessibility of *in vitro* and *in vivo* experimental systems to study lymphatic

biology have highlighted a more dynamic role for the lymphatic vasculature in metastatic tumour spread [110-112].

The lymphatic capillary is a thin walled structure consisting of single layer of endothelial cells lacking inter -endothelial tight junctions. They do not have smooth muscle cells and a basement membrane as the blood capillaries. The main function of lymphatic vasculature is regulation of tissue fluid homeostasis, antigen collection from peripheral tissues, and mediate immune cells such as antigen-presenting dendritic cells from the periphery to lymph nodes. It also provides a unidirectional transport system that relies on skeletal muscle contraction and respiratory movement for the transport of lymph. This is a process which occurs in adults only during pathological conditions such as inflammation, tissue repair or tumour growth. Many molecular factors that contribute to lymphangiogenesis have been studied recently, among which VEGF C and D bind to the VEGF-3 receptor expressed on the endothelial cells of the lymphatic vessels [112-114].

More recently, several factors with pro lymphangiogenic activity have been identified. These include hepatocyte growth factor, which binds to the c-met receptor, angiopoietin-1 together with its endothelial cell–specific receptor Tie-2, FGF1 and -2, PDGF, insulin-like growth factor-1 and -2, and endothelin-1 [115-119].

Tumour-induced lymphangiogenesis is mediated by lymphangiogenic growth factor produced and secreted by the tumours. The role of VEGF-C and VEGF-D in cancer progression has been extensively studied. The overexpression of either of the two factors in tumours significantly increased tumour-associated lymphatic vessel growth (mainly at the tumour margin) and increased incidence of lymph node metastasis. The lymphangiogenic growth factors along with increasing vessel density, also enlarge and dilate vessel size. VEGF 2 receptor is important in this process, while VEGF 3 receptor is involved in endothelial cell sprouting [119-124].

1.4.5. Cytomegalovirus and cancer

Emerging evidence indicate that cytomegalovirus (CMV), which is not considered as an oncogenic virus, is highly present in several types of cancer. CMV belongs to the family *Herpesviridae* and the subclass *Betaherpesviridae.* CMV remains latent in the body for the life time of its host after a primary infection without causing any clinical disease in healthy individuals. It can reactivate from time to time, but is kept under control by the immune system However, in immunocompromised individuals CMV infection may be life threatening; it causes major morbidity and mortality in stem cell and organ transplant patients as well as in AIDS patients. It is also a common cause of birth defects in children who suffer from a congenital infection. Emerging evidence suggest that the virus is detected in high prevalence in tumours of different origin. Over 90% of glioblastoma, neuroblastoma, medullblastoma, colon, breast and prostate cancers are positive for CMV proteins and nucleic acids. The virus is also detected in lymph node and brain metastases of colon and breast cancer patients. However, it is rarely found in healthy tissue surrounding the primary tumour. Although HCMV is found in lymphnode and distant metastases, the potential virus related mechanisms of metastasis promotion are not understood [125, 126].

1.4.5.1. CMV in tumour dissemination

HCMV is known to play a role in tumour dissemination. For example, HCMV infection of neuroblastoma cells leads to enhanced tumour cell adhesion to endothelial cells resulting in the disruption of EC monolayer integrity. This leads to enhanced neuroblastoma invasiveness and higher trans-endothelial migration.[127] [128]. Studies also show the role of HCMV in EMT related to metastasis. A study by Shimamura et al examined the role of HCMV in the induction of TGF-β and its role in EMT. Upon infecting human renal tubular epithelial cells were *in vitro* it was observed that these cells underwent morphologic and transcriptional analogous related to EMT. Also, TGF-β and MMP-2 expression was induced. HCMV IE proteins probably control this process as their overexpression summarized these effects and targeting late gene expression did not inhibit these changes.

Several factors involved in EMT are similarly modulated by HCMV. This includes growth factors and signalling pathways. Further studies are required to comprehend the role of HCMV infection in EMT transition and metastasis formation.

1.5. Cancer Immunology

Metastasis is the key factor for cancer related deaths. Thus, understanding the mechanism of tumour dissemination is the central factor of cancer research. In cancer patients, the disseminated tumour cells are detectable in the peripheral blood as circulating tumour cells (CTC's) while in the lymph nodes they are detected as disseminated blood cells (DTC's). Hence the identification and characterization of these cells have resulted in perceptions of the molecular mechanisms of metastasis [129].

Cancer cells that exist in the primary site of a tumour are immune protected, cells which exit this site and enter circulation are compromised and vulnerable to immune surveillance, hence the survival of these cells are essential for metastatic spread. Thus, immune escape mechanisms are required for cell survival [129].

Immune escape mechanisms or immunoediting are important aspects for cancer cell survival. Immunoediting is a process which comprises of immune surveillance and tumour progression. It consists of three phases leading to cancer progression. The first phase is called elimination, here neoplastic cells are contained and destroyed by innate and adaptive immune cells. The second phase equilibrium is attained following escape the neoplastic cells escape from elimination and their interactions with immune cells reach an equilibrium with the immune system exerting a constant pressure. Hence even though the immune system wants to prevent the cells from progressing, it unwillingly contributes by selective clonal selection. This leads to the third stage, the escape phase, where the colonies which survived the previous phases, gain the ability to grow in an immune competent environment [130-132].

Tumours which escape immune evasion acquire resistance to immune factors, for example interferon gamma. Insensitivity to interferon gamma, enhances tumour resistance to immune attacks. Also, tumours evade immune cells either by shedding or restricting presentation of their ligands for recognition by NK cells and cytotoxic T lymphocytes. The tumours also downregulate other factors that elicit a tumour-immune response, for example pro inflammatory cytokines and chemokines [133-138].

1.5.1. Immune surveillance

NK cells and macrophages are the most studied cells with respect to tumour suppression and surveillance. NK cells are an important part of the innate immune system and they play a major role in defence against tumours and viruses. The interactions of these cells with tumours is mediated by a network of receptors and ligands including the major histocompatibility complex (MHC) class 1- related inhibitory molecules. Inhibition of NK cell signalling leads to tumour lysis through cytolytic granules release and apoptosis induction. These effects of the NK cells are decreased in CTC positive patients with metastatic colon, breast and other types, compared to CTC negative patients [138-141].

Macrophages, along with NK cells have an important role in controlling metastatic progression. Macrophages are vital for antibody dependent phagocytosis of tumours. This process is mostly governed by macrophages in the liver. A study by Denève et al showed comparisons of CTC counts between peripheral and mesenteric blood samples in patients with colorectal cancer confirmed that a significant proportion of the viable CTC population seem to be filtered and trapped in the liver. Thus, these findings highlight the importance of the liver microenvironment in mediating the outcome of interactions between tumour cells and immune cells. This often promotes tumour-cell death and sometimes facilitates DTC survival and growth [142-144].

1.5.1.1. Immunoediting

The immunological elimination of tumour antigen is driven by T- cell recognition, which is the chief principle of cancer immunoediting. Studies have validated this finding, one study on genetic mapping of a highly immunogencic and unedited sarcoma derived from methylcholanthrene (MCA)-treated Rag2−/− mice, to determine its mutational landscape showed that cancer immunoediting is the consequence of a T-cell-dependent immunoselection process which leads to outgrowth of tumour cell clones that lack immunodominant rejection antigens displaying reduced immunogenicity. A study by DuPage et al reached a similar conclusion. Studies also show the involvement of innate immunity in the immunoediting process. They showed that NK cells (apparently activated by local amplification of endogenous IL-12) can produce IFN-γ which in turn induces activation of $CD45^{+}CD11b^{+}MHCII^{hi}CD206^{lo}Ly6C^{lo}$ M1 macrophages. These act as important effectors of cancer immunoediting. Thus, these results show that the degree of immunocompetence of the host plays an important role in the extent to which a tumour undergoes immunoediting. [5,16].

1.5.1.2. Elimination:

Many reviews have described and summarized the mechanisms that take place in the elimination phase. The role of host recognition molecules such as NKG2D; IFN-γ, perforin, effector molecules like Fas/FasL, and TRAIL; and an intact lymphocyte compartment in protective anti-tumor immunity, are well recognized. Both type I (IFN-α/β) and type II interferons (IFN-γ) are essential for development of anti-tumour immune responses but play diverse roles in the cancer immunoediting process, while IFN-γ targets both tumour cells

and hematopoietic cells and tumour cells, IFN- α/β acts primarily on host cells. Recently, two studies showed that type I IFNs are mandatory for initiation of the early anti-tumour response and act on CD8α/CD103$^+$ DCs to enhance cross-presentation of tumour antigens to CD8$^+$ T cells [18,19]. Type I IFN sensitivity was not required for tumour rejection in macrophages, NK cells and granulocytes, all of which express type I IFN receptors.

1.5.1.3. Equilibrium

Adaptive Th1-like immunity plays an important role in the equilibrium phase and this was described in two studies, one using an immune-mediated dormancy model of fibrosarcoma and a follow up study using the same mouse model of MCA- induced fibrosarcoma and p53 mutant tumours. Both showed that immune-mediated tumour dormancy may be a very lengthy process. Significantly, the balance of IL-12 promoting elimination, and IL-23 (sharing the common subunit IL-12 (p40) maintains tumours in equilibrium and promotes persistence. Along with a minor tumor-promoting role for IL-10 many other pathways (e.g. IL-4, IL-17A, TNF, IFN-αβ) were shown to be dispensable for this phase.

Tumours that escape go on to metastasize. Specific mechanisms are involved in this process.

1.5.2. Immune evasion mechanism

As mentioned before, CTCs leaving the immunosuppressive primary tumour microenvironment are exposed to the active immune surveillance mechanisms. In addition, the possibility that CTCs will be lysed by tumour-specific immune cells increases significantly outside the immunosuppressive reserve of the tumour since peripheral immune cells are more numerous than CTCs. Hence the circulatory system is considered a hostile environment for cancer cells. Primary tumours are predicted to shed thousands of cells into the bloodstream every day, but evidently only a very small percentage develop the ability to grow into distant metastases supports this assumption. However, studies have identified new pathways through which CTCs might evade or survive encounters with immune cells. The most established mechanism of tumour evasion includes the ones previously described (NK cells and macrophages), CD 47 signalling, FAS/FASL signalling and hypoxia induced apoptosis [145].

1.5.2.1. MHC molecules and NK-cell ligands:

MHC I molecules that expressed on the surface of basically all nucleated cells present peptide epitopes that are processed from intracellular proteins for examination by immune cells. Thus, presentation of tumour-associated antigens (TAAs) to T-cell receptors (TCR) in the context of MHC I molecules is crucial for initiation of an adaptive CD8$^+$ CTL response. Hence, downregulation or entire loss of MHC I expression at the cell surface is a mechanism used by tumour cells to ‹hide› from CTLs and thereby evade death. As a standby to counteract this mechanism, NK cells become activated when MHC I molecules are under expressed or absent. Therefore, to escape both NK-mediated and CTL-mediated cytotoxicity, CTCs have to find a way to present MHC I molecules without presenting TAAs [146-149].

Cytokeratin 8 (CK8), along with its heterodimeric partners CK18 and CK19, have been shown to inhibit MHC I interactions with TCRs on CD8$^+$ CTLs. Overexpression of these cytokeratins has long been detected in malignant tissues. This mechanism demonstrates how cancer cells develop new methods of immune evasion, and that interfering with MHC-mediated antigen presentation seem like a critical approach to immune escape [150, 151].

1.5.2.2. FAS/FASL induced apoptosis

This apoptotic pathway is highly important to immune evasion. The transmembrane receptor FAS can initiate apoptosis, and activation of this receptor on T cells via binding to FASL is a suggested mechanism of tumour-mediated immunosuppression in several malignancies. For example, histopathological analyses have shown that FASL is upregulated in metastases compared to the primary tumour in patients with melanoma or colorectal cancer. Also, in patients with breast cancer, upregulation of FASL has been associated with increased apoptosis of T cells. Hence FASL expression on tumour cells may actively induce apoptosis in immune cells. Vice versa, tumour cells that express FAS will most likely be vulnerable to apoptosis evoked by tumour-specific immune cells, which can also express FASL. Hence, instantaneous loss or downregulation of FAS and upregulation of FASL on tumour cells might add to tumour evasion of immune-mediated cytolysis and increase the prospective for metastatic progression [152-156].

1.5.2.3. CD47-mediated signalling

Exhaustive studies by Irving Weissman and colleagues have emphasized the role of the leukocyte surface antigen CD47 in cancer, mostly, in cancer-cell evasion of phagocytic clearance. CD47 binds to its ligand signal-regulatory protein α (SIRPα, also known as macrophage fusion receptor), expressed on macrophages and dendritic cells, subsequently inhibiting phagocytosis by these cell types. Therefore, upregulation of CD47, an antiphagocytic 'don't eat me' signal, might confer CTCs with a non-immunogenic profile by allowing them to escape the consequences of cell-damage-induced upregulation of pro-phagocytic signals and, thus, the immune sequelae evoked after CTC recognition in the context of adaptive immunity. Steinert *et al* have associated the gene-expression profiles of primary tumours and CTCs from patients with colorectal cancer. Notably, CD47 was the only gene upregulated in CTCs versus the matched primary tumours, signifying a survival advantage conferred by CD47 expression for peripheral blood CTCs. Therefore, these findings along with others propose that CD47 is part of a potential metastasis-initiator cell signature, but functional analysis is required to describe the exact role of CD47 expression on CTCs [157-162].

1.5.2.4. Hypoxia-induced immune escape

Various genes are expressed during EMT, in cancer-stem cells, or in response to hypoxia. They have been shown to be upregulated in CTCs, and many of the encoded proteins can control the immune response. Specific metabolic and molecular changes enable DTCs to adapt to and survive in a microenvironment with a lower oxygen concentration, such as the bone marrow. Evidence investigating HIF-1αexpression in CTC's and functional studies of DTC's on bone marrow patients with lung, breast and prostate cancer show that many CTCs and DTCs exhibit a hypoxia-associated phenotype, and can adapt well in hypoxic condition. For example, upon hypoxic stress, glucose-regulated protein 78 (Grp78) is upregulated in cell lines established from the bone marrow of patients with cancer, and expression of Grp78 is linked with mesenchymal characteristics and poorly differentiated primary breast and lung tumours. Ongoing studies are investigating if adaptation to hypoxia also promotes CTC and/or DTC evasion of immune cells. Nonetheless, the hypoxia-resistant phenotype of DTCs has implications for immunotherapeutic strategies [163-165].

1.5.2.5. Metastasis promotion by immune cells

Studies show that metastasis can be supported by immune cells. Immune cells can, hence, be regarded as both protagonists and antagonists in the metastatic process [166-168].

1.5.2.6. Promotion of CTC seeding

Preliminary studies of CTCs have established a positive correlation between an acute inflammatory condition and formation of metastasis in the target organ of metastatic spread. For instance, using an allergic pulmonary inflammation model indicated that for CTCs to extravasate and form tumour filiae, take advantage of the augmented vascular permeability and adhesion molecules expression at the site of metastasis. They also showed that for CTC's to metastasize to the lung, they required the presence of $CD4^+$ cells at the site of metastasis. Along these lines, data from a murine colorectal cancer model indicated a positive correlation between CTCs formation and serum levels of IL-17A, a proinflammatory cytokine. In addition, the presence of IL-17A augmented tumour-cell motility. This occurs by triggering MMP-9 expression in CTCs, hence possibly supporting CTC mobilization and extravasation [169, 170].

Put together, escape from and variations in peripheral immune responses outside the local tumour milieu, are critical steps in metastases development.

1.6. C/EBPβ

C/EBPβ is a protein belonging to the C/EBP family [171, 172]. The family consists of six transcription factors from C/EBPα to C/EBPζ in total and is characterized by a basic leucine zipper at the C-terminus required for binding and dimerization. The family of proteins regulate different gene expressions involved in cell differentiation, proliferation inflammation and metabolism.

C/EBPβ can form heterodimers with members of the C/EBP family, such as C/EBPα, C/EBPγ and C/EBPδ, along with other transcription factors like Sp1or CREB1. It can also bind as a homodimer to some DNA regulatory regions thereby controlling the expressions of various target genes.

As a transcription factor, C/EBPβ interacts with several target genes and is required for a variety of biological processes, such as, granulopoiesis, adipogenesis, muscle repair, embryogenesis, and osteoporosis. It is involved in controlling autophagy, cell growth and antibacterial defence, along with regulating insulin level and insulin receptors expression. C/EBPβ is also involved in regulation of multiple genes responsible for immune and inflammatory responses. Evidence show its binding to cytokine coding genes such as IL-4, IL-6, IL-5 and TNFα. It is also responsible for activation and terminal differentiation of macrophages, an important immune cell subtype.

Our studies emphasize the involvement of C/EBPβ in breast cancer here its gene is usually non- mutated. A few rare mutations that have been found are questioned in its contribution to epithelial tumours. However, C/EBPβ might be amplified in a small subgroup of breast neoplasia, described as lobular carcinoma in situ. Elevated levels of C/EBPβ mRNA are linked to metastatic breast cancer, higher tumour grade and overall worse prognosis [171, 172].

1.7. Cancer progression and EMT

Epithelial-Mesenchymal transition is a process by which epithelial cells transform into mesenchymal cells. This occurs due to the loss of cell polarity and cell-to-cell adhesion molecules. This process is important in wound healing and embryogenesis. The reverse process mesenchymal epithelial process is also essential for various organ developments. It is also known to be involved in cancer progression and metastasis. Epithelial cells are single or multilayer cells with various functions. They depict apical-basal polarity and through specialized intracellular junctions, adhere and communicate with adjacent cells. Their position and interaction of the basement membrane proteins with integrins help define their physiology. The transition of the cells follows certain hallmarks and patterns. The plasticity of the epithelial phenotype enables cell transition through multiple EMT and MET rounds.

EMT increases the invasive phenotype of the cancer cells. They lose their cell-cell adhesion molecule, E-cadherin and the basement membrane. TGF-beta is a major factor that induces this property in tumour cells when it acts on activated RAS- expressing cells, leading to EMT and inhibition of apoptosis[173]. Evidence suggests that activated platelets have a direct contribution to the invasive phenotype of the cancer cells at the primary tumour site. In breast carcinoma, higher levels of TGF beta 1 and TBRII can be found. Similarly NOTCH and WNT signaling are associated with CSC's. In colon cancer, nuclear beta catenin is visible in scattered tumours [174]. The expression of TGF- beta varies with different cell types, thus understanding and quantifying the process is difficult [173, 175, 176].

There are three types of EMT based on the physiological context. Type 3 EMT leads to cancer progression and cancer stem cell properties. Type 1 EMT is the differentiation of epithelial cells to mesenchymal cells with no prior history of transition. Type 2 EMT is a process where cells have already undergone transition followed by reversion and initiation of a new EMT. Similarly, following dissemination, cancer cells revert to epithelial cells through MET and secondary carcinomas are generated having similar phenotypes. Pro invasive function of cancer cells is attributed to the expression of avβ3 integrin that is increased due to EMT [177, 178].

Various transcription factors like SNAIL, TWIST and ZEB have prominent roles in EMT and cancer progression. They have different profiles and functions based on the cell type. TWIST 1 down regulates epithelial gene expression and enhances expression of mesenchymal genes. SNAIL 1 and 2 has a similar role. Other transcription factors such as forkhead box (FOX) and GATA family and growth factors such as VEGF and FGF are also involved in EMT regulation [173, 175].

1.8. Death mechanisms and survival

Sudden cardiac death, a major problem worldwide is an unexpected natural cause of death due to cardiac failure. The time usually accounts for less than an hour from onset of symptoms without any prior condition would appear fatal. Such a quick death is attributed to cardiac arrhythmia. It is the most common and often, the first appearance of coronary artery disease. It is also the cause of 50% of cardiovascular deaths. The incidence increase with age in both men and women.

Sudden cardiac death may perhaps be considered as an electrical accident since, even though many patients have transient events that could influence the initiation of ventricular tachycardia or ventricular fibrillation and many individuals have anatomic and functional substrates favourable to developing a life-threatening ventricular tachyarrhythmia, only a relatively small percentage develop sudden cardiac death. This interplay between the anatomic and functional substrates (such as CAD, cardiomyopathy dilated and hypertrophic) modulated by the

transient events (like neuro& endocrine drugs, ischemic & reperfusion injury) that disturb the balance, and the impact of all 3 on the underlying potential arrhythmia mechanisms possessed by all hearts triggers sudden cardiac death. For instance, the combination of the 3 factors, i.e., coronary artery disease, scarred myocardium, and hypokalaemia, might be sufficient to provoke a ventricular tachyarrhythmia, causing sudden cardiac death in a patient had pre-existing re-entry pathways in the ventricular myocardium, likely due to an old infarction.

Variations in the anatomic substrate can modify the susceptibility of the myocardium to that of transient initiating events. For example, experimental studies show that arrhythmogenic response in a hypertrophied myocardium, and a healed myocardium post a myocardial infarction, is greater than normal tissue to the same extent of acute ischemia.

Another factor that can modulate some of the effects of acute coronary occlusion and reperfusion is catecholamine release along with reduction in sympathetic action with drugs presented to the pericardial sac to super fuse sympathetic nerves results in prevention of ventricular arrhythmias. Acute ischemia alone, involving a sufficiently large area of myocardium in an otherwise normal ventricle, can cause ventricular fibrillation without interplay with other factors though it is interesting to reflect the many balloon angioplasties performed and the infrequent occurrence of ventricular fibrillation during that procedure. Possibly the duration of ischemia is too short and inefficient to initiate. Re-entry along with regional changes in automaticity, as well as triggered activity due to afterdepolarisations, are probably important mechanisms to trigger ventricular fibrillation. Reperfusion can also be arrhythmogenic.

Another mechanism for cardiac arrest could be due to severe asystole, bradycardia, or pulseless electrical activity (electromechanical dissociation). In severely diseased hearts, this is more common and probably represents more global myocardial dysfunction.

Another major electrophysiological feature accountable for the initiation of ventricular fibrillation seems to be electrical heterogeneity. A heart that is entirely homogeneous electrically, i.e., all cells are at the same stages of repolarization and depolarization and conduct normally without block or delay, most probably does not develop ventricular fibrillation.

Essentially, even under normal circumstances these conditions do not exist, since various cell types, e.g. ventricular muscle versus Purkinje fibres, exhibit different refractoriness, action potential characteristics, and conduction velocities. But when heterogeneity becomes extreme, for instance, if one region of the myocardium exhibits ischemia-induced conduction block or delay differing from neighbouring regions, or when there is an unequal stretch or regional dysfunction causing regional electrophysiological alterations, the stage becomes set for development of ventricular fibrillation. These changes can be provoked by anatomic/functional substrates, transient initiating events and can moderate basic arrhythmia mechanisms of automaticity, re-entry and triggered activity to provoke ventricular arrhythmia.

1.8.1. Mechanism of death in cancer

Cachexia is a major recognized syndrome and a critical factor for cancer death. It is a multi-step process involving skeletal muscle and adipose tissue atrophy, leading to weight loss. It is attributed to poor physical function, lifestyle and prognosis in cancer patients. The classical clinical feature of cachexia is weight loss in adults and failure of growth in children. Various factors such as anorexia, inflammation, insulin resistance and muscle breakdown are also associated with cachexia. This problem, common to cancer patients has not been clearly defined.

The steps involved in this process include; widespread metabolic changes, proinflammatory signals arising from tumour cells and systemic inflammation in the host. Loss of body weight is a key signal and a loss greater than 5-10% is the defining limit. A weight loss above 30% results in death. The degree of loss on prognosis and outcome has not yet been clearly defined.

Until a while ago cancer cachexia was defined as a wasting syndrome that involves loss of fat and muscle, and it may or may not be attributed directly to tumour factors. This is due to the host's unusual immune response to it. More recent definitions state the cancer cachexia is a metabolic syndrome that is related to an underlying illness. It is characterized by muscle loss with or without loss of fat mass, thus emphasizing a unique property which is muscle wasting thereby, establishing it as a hallmark of cachexia. The metabolic syndrome is the result of protein synthesis, lipid metabolism and its degradation. These factors eventually result in cachexia. The changes occur due to an infection rather than starvation. These changes are complex and depend on various factors.

Decreased muscle strength is useful as a diagnostic criterion for patients with cancer cachexia, it is also used to differentiate between other forms of anorexia and the ones related to cancer. Patients who lose weight have a systemic inflammatory response [179, 180].

In a cancer patient, the main reason for weight loss is attributed to total loss of skeletal and adipose tissue mass. An increase in intracellular proteolytic activity is the reason for weight loss in the body. A complete catabolism of muscle tissue takes place leading to net loss of mass. Normal weight loss is due to depletion of adipose stores owing to starvation and the greatest contributor for cachexia is the ATP dependent ubiquitin pathway.

Clinical consequences of cachexia are determined by various factors such as host tumour interactions and metabolic syndromes, both leading to endpoints resulting eventually in cachexia. Each of these factors involves various trivial interactions. For instance, when a tumour is formed, the host initiates immune responses to deal with the tumour. An acute phase response is launched leading to the formation of a systemic inflammation.

The inflammatory cytokine response of the host against the tumour could possibly drive the cachexia process. Pro inflammatory cytokines include IL 6, TNF α and IL 1. It is not clear if the cytokine production is from the tumour or the host cell. A theory suggests that, it could be either produced by the tumours or occur due to the host's immune response against the tumour as a source of an acute phase protein response seen in various malignancies and in cachexia. Mouse models show that the systemic inflammatory response against the tumour correlates with weight loss and an interchange between IL1β and IL 6 inside the tumour microenvironment.

The tumour produces both pro cachectic and pro inflammatory factors that lead to an immune response. The pro cachectic factors include proteolysis inducing (PIF) and lipid mobilizing (LMF) factors. PIF and TNF α are the major competitors in skeletal muscle atrophy in patients with cachexia. Both of them increase protein degradation through an ubiquitin – proteasome pathway and downregulate protein synthesis through the phosphorylation of the eukaryotic initiation factor 2 alpha [179, 181, 182].

PIF has been found in the urine samples of weight loss patients having breast, colon, lung and ovarian cancer. In animals, the signaling occurs though NFκβ and STAT 3 pathways. If these pathways are incited, they lead to proteolysis in the muscle through a ubiquitin-proteasome pathway. In the hepatocytes, it concludes in the production of IL-6, 8 and CRP. LMF is found in patients with cancer related weight loss and not in patients with stable weight [179, 183].

Changes in lipid metabolism are due to lipolysis and are driven by LMF along with the tumour and a host factor known as zinc-2-alpha glycoprotein. This has a direct effect and sensitizes adipocytes to lipolytic stimuli resulting in an overexpression in cachexia. Another implicating factor is the resting energy which disrupts and dis-regulates the energy regulation. This has been found to be higher in cancer patients compared to non-cancer patients. A theory for this could be an altered gene expression in the mitochondria of the uncoupling proteins resulting in uncoupling of respiration from ATP production. This leads to loss of energy as heat.

Thus, the metabolic changes leading to cachexia are due to the interplay between tumour host interaction factors [179, 183].

1.8.2. Red blood cell distribution width (RDW)

Red blood cell distribution width or anisocytosis refers to the uneven size of the red blood cells with a higher RDW than normal. It is usually denoted in combination with red blood cell corpuscular volume in diagnosis of chronic inflammatory status in the body. Anisocytosis is commonly found in anemia and other blood disorders [184, 185].

Besides many blood disorders, RWD is associated with various acute and chronic cardiovascular diseases such as peripheral artery disease, acute coronary syndrome and ischemic cerebrovascular disease. Many studies have a shown an interesting relationship between carotid atherosclerosis or stroke. A reason for consistent increase in RDW in CVD is attributed to active stimulation of erythropoiesis by erythropoietin (EPO) a hormone, secreted during hypoxic conditions. This promotes the release of enflamed RBCs from the bone marrow [186]. Another hypothesis for high RBC could be due to a slight reduction in RBC turnover. Since size of RBCs gradually reduces with ageing of the cells, a decreased rate of RBC turnover would allow smaller cells to continue longer in circulation.

1.8.3. Survival

Post-secondary malignancies, CVD is the foremost cause of morbidity and mortality among cancer survivors. CVD risk factors are predominant in cancer patients. A study by Mertens et al[6] showed that among childhood cancer survivors, cardiovascular events are the principal non-malignant cause of death. It is responsible for a higher risk of death, about 7-fold high, among these patients when compared to their peers. After effects of cardiotoxic cancer therapy is thought to be the fundamental cause for this. Alternatively, a study by Enright and

Krzyzanowska[10] reiterated the necessity for precise individualized cardiovascular disease prevention program for cancer survivors. They showed that the subpar control provided for traditional measure of risk factors like cholesterol monitoring and blood pressure among survivors.

Recent developments indicate an increasing interest in a hypothesis for development of CVD in cancer patients. This proposed that CVD development in cancer patients takes place when they are exposed to a series of sequential or simultaneous events that together make them more susceptible to cardiovascular reserves and ultimately result in death. Another risk factor for pathogenesis of CVD is psychological distress in non-cancer populations. Put together the multiple-hit hypothesis has been conceived.

Cancer treatments include chemo, radiation, immuno or hormone targeted therapies or a combination of these. Some amongst them are cardiotoxic. For instance, for lymphoma or breast cancer treatments, chemo with anthracyclines as well as radiation therapy to the chest are cardiotoxic. These can lead to a reduction in cardiovascular reserves and eventually different sets of CVDs ranging from benign to possibly fatal. CVDs associated with cancer treatment can occur within few days, months or years. They include arrhythmias, myocardial infarction, thrombosis, congestive heart failure and cardiomyopathy.

Lifestyle factors also contribute to process. Due to cancer treatments patients might develop an unhealthy life style which includes physical inactivity and weight loss. This might lead to reduction of CV reserves and augments CVD risk and death. Psychological distress another important risk factor, whose presence is a bad for health outcome of patients. independent of traditional biomedical risk factors, depression symptoms, fatigue and anxiety have shown to forecast CVD onset and prognosis in patients with established CVD. This was validate based on a meta-analysis of 20 studies which showed the value of anxiety prediction for coronary heart disease occurrence in formerly healthy individuals. It showed that there is 26% higher risk of coronary heart disease development and a 48% increase in risk of cardiac death among anxious individuals.

Thus, research on adapted multiple-hit hypothesis for CVD development among cancer patients could contribute to advances regarding their care. An immediate necessity for CVD preventive procedures to reduce the delayed adverse effects of cancer therapies such as radiation and chemotherapy and early intervention could possibly help improve CVD's risk profile.

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