RAQUEL SARMENTO MATEUS

REMODELAMENTO DA MATRIZ EXTRACELULAR E MECANISMOS DE RESPOSTA AO ESTRESSE INDUZIDOS PELO PEPTÍDEO DA LAMININA C16 EM CÉLULAS EXPOSTAS À HIPÓXIA QUÍMICA OU BAIXA TENSÃO DE OXIGÊNIO

EXTRACELLULAR MATRIX REMODELING AND STRESS RESPONSE MECHANISMS INDUCED BY LAMININ DERIVED PEPTIDE C16 IN CELLS EXPOSED TO CHEMICAL HYPOXIA OR LOW OXYGEN TENSION

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Orientador: Prof. Dr. Ruy Gastaldoni Jaeger

Co-orientadora: Profa. Dra. Lynne-Marie Postovit

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RESUMO

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O microambiente das células tumorais desempenha importante papel no processo de tumorigênese. Entre diversos componentes, a laminina, uma glicoproteína da membrana basal, pode sofrer clivagem por ação de metaloproteinases (MMPs), e seus fragmentos podem ser bioativos. Um desses fragmentos, é o peptídeo C16, que promove atividades pró-tumorais, como por exemplo, a formação de invadopódios (protrusões “finger-like” da membrana com atividade proteolítica), que células tumorais usam para iniciar invasão. Os efeitos observados do peptídeo C16 foram adquiridos em condição de normóxia (tensão normal de oxigênio). No entanto, o microambiente tumoral possui regiões em baixa tensão de oxigênio (hipóxia), uma condição que promove maior estresse no tumor, ao estabilizar o fator induzido por hipóxia (HIF1-α), o qual influencia diversos aspectos da biologia tumoral. Com isso, este estudo investigou a regulação de mecanismos de estresse e de invadopódios pelo peptídeo C16 em células de fibrosarcoma humano (HT1080), que foram submetidas à hipóxia, induzida por cloreto de cobalto (hipóxia química); ou por baixa tensão de oxigênio, em câmara de hipóxia. Células HT1080 na presença do peptídeo C16, e em fenótipo hipóxico (confirmado pelos altos níveis proteícos de HIF1-α), ou em normóxia, aumentaram a degradação de um substrato fluorescente, possibilitando a verificação da atividade de invadopódios. Além disso, também notou-se uma aparente maior dispersão das proteínas-chave de invadopódios, cortactina e MT1-MMP, nesses grupos em relação aos controles. Observou-se também uma ativação de MMP-2 pelo peptídeo, integrando o aspecto degradativo. Surpreendentemente, células na presença do peptídeo C16 tem seu tamanho reduzido, e o número de células é menor após 3 dias. O peptídeo C16 também reduziu a síntese de proteínas nas células, em condições de normóxia e hipóxia. Esses fatores levaram á verificação da via de mTOR, o que demonstrou diversas proteínas desta via reduzidas e levou ao desaparecimento da proteína ribossomal S6, pelo peptídeo C16 em hipóxia. Esses resultados demonstram um efeito estressante do peptídeo, promovendo a adaptação celular por regulação de mecanismos de estresse e estimulando o remodelamento da matriz extracelular por MMPs e ao induzir a formação de invadopódios.

Palavras-chave: Hipóxia. Laminina. Fibrossarcoma. MMPs. Via de mTOR. Invadopódio.

ABSTRACT

Mateus, R. S. **Extracellular matrix remodeling and stress response mechanisms induced by laminin derived peptide C16 in cells exposed to chemical hypoxia or low oxygen tension.** 2021. **Ph.D Thesis** (Cell and tissue biology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2021.

The microenvironment of tumor cells plays an important role in the tumorigenesis process. Among several components, laminin, a basement membrane glycoprotein, can undergo cleavage by the action of metalloproteinases (MMPs), and its fragments can be bioactive. One of these fragments is the peptide C16, which induce pro-tumor properties, such as the formation of invadopodia (finger-like membrane protrusions with proteolytic activity), which tumor cells use to initiate invasion. The peptide C16 effects previously observed were acquired under normoxia condition (normal oxygen tension). However, the tumor microenvironment has regions of low oxygen tension (hypoxia), a condition that promotes greater stress on the tumor, by stabilizing the hypoxia-inducible factor (HIF1-α), which influences several aspects of tumor biology. Thus, this study investigated the regulation of stress and invadopodia mechanisms by the peptide C16 in human fibrosarcoma cells (HT1080), which were exposed to hypoxia, induced by cobalt chloride (chemical hypoxia), or by low oxygen tension, in a hypoxia chamber. HT1080 cells in the presence of peptide C16, and in hypoxic phenotype (confirmed by high protein levels of HIF1-α), or in normoxia, increased the degradation of a fluorescent substrate, enabling the verification of invadopodia activity. In addition, an apparent greater dispersion of key proteins from invadopodia, cortactin and MT1-MMP was also noted in these groups compared to controls. An activation of MMP-2 by the peptide was also observed, integrating the degradative aspect. Surprisingly, cells in the presence of peptide C16 are reduced in size, and the number of cells is lesser after 3 days. The peptide C16 also reduced protein synthesis in cells under conditions of normoxia and hypoxia. These factors led to the verification of the mTOR pathway, which demonstrated several reduced proteins and led to the disappearance of the ribosomal protein S6, by the peptide C16 in hypoxia. These results demonstrate a stressful effect of the peptide, promoting cellular adaptation by regulating stress mechanisms and stimulating extracellular matrix remodeling by MMPs and inducing invadopodia formation.

Keywords: Hypoxia. Laminin. Fibrosarcoma. MMPs. mTOR pathway. Invadopodia.

1. INTRODUCTION

Cancer is a designation for malign tumors, or in other words, abnormal growth of cells (neoplasia) that disrupts the tissue or organs in which they are located and may also be capable to migrate into other sites of the body (metastasis) promoting systemic alterations and lead to mortality (Compton, 2021; WHO, 2021).

Over the last decades, cancer has been one of the leading causes of mortalities worldwide. For instance, data reported from around the world shows that cancer is among the main diseases, hence, in 2019, 57 countries had cancer as a leading cause of death (Bray *et al.*, 2021; Sung *et al.*, 2021). Moreover, according to the World Health Organization, in 2020 around 10 million people died of cancer diseases around the globe (WHO 2021).

As a prominent condition, cancer has been constitutively studied with the aim to achieve ways to treat it, as mortality related to metastasis can be higher than 90% (Neophytou *et al.*, 2021). Through the years, great knowledge about the tumors' characteristics has been gathered, stating that cells go through a tumorigenic process, in which they acquire: greater proliferative potential, evasion of programed cell death (apoptosis), promotion of vascularity (angiogenesis), and ability to invade and metastasize (Hanahan and Weinberg, 2011; Hanahan and Coussens, 2012).

Furthermore, the environment surrounding the tumor cells also plays a very important role in the tumorigenesis process. The tumor microenvironment (TME) is a complex niche, where multiple molecules and different type of cells interact and exchange components (Hanahan and Weinberg 2011; Balkwill, Capasso, and Hagemann 2012; Quail and Joyce 2013). Some of these elements are combined into the Extracellular Matrix (ECM), which composes a three-dimensional macromolecular network of collagen, proteoglycans and glycoproteins, and therefore influences many aspects of tumorigenesis and tumor progression (Kalluri, 2003; Frantz, Stewart and Weaver, 2010; Hanahan and Coussens, 2012).

In some regions, such as close to epithelial and connective tissue and surrounding vessels, the ECM has a more specific arrangement, primarily to give support and anchor

the cells nearby. This specialized part is known as basement membrane, and consists mainly in laminin and collagen IV, but also have perlecan and nidogen. Laminin is a trimeric-cruciform shaped glycoprotein, consisted of 3 polypeptide chains called α, β and γ. Around 16 isoforms have been identified, ranging between 400-900 kDa. Moreover, cells attach to laminin by receptors, such as integrins (Kalluri, 2003; Yurchenco, 2011; Aumailley, 2013; Mak and Mei, 2017; Hallmann *et al.*, 2020).

In the TME, cancer cells can acquire traits that lead them to invade the surroundings and migrate to new sites. In order to do that, cells promote the cleavage of ECM molecules, such as laminin. This remodeling is carried out by enzymes, mostly matrix metalloproteinases (MMPs), and this process generates fragments and peptides that have biological effects and support the cells to acquire a more aggressive phenotype (Schenk and Quaranta, 2003; Freitas *et al.*, 2004; Paz, Pathak and Yang, 2014). Previous studies of fragments from laminin-111 demonstrate the effect of these peptides promoting tumorigenic behaviors as: migration, invasion, secretion of proteases, invadopodia formation and altering signaling pathways (Kuratomi *et al.*, 2002; Freitas *et al.*, 2007; Nascimento *et al.*, 2011; Kikkawa *et al.*, 2013; Siqueira *et al.*, 2016; Caires-dos-Santos *et al.*, 2020).

One bioactive peptide released by the cleavage of the γ1-chain of laminin-111, is peptide C16, which has been shown to induce adhesion, migration, proteolytic activity and invadopodia formation and activity, by triggering related signaling pathways, such as the increase of the phosphorylation on ERK 1/2 and Src proteins (Kuratomi *et al.*, 2002; Pinheiro *et al.*, 2011; Kikkawa *et al.*, 2013; Siqueira *et al.*, 2016; Smuczek, 2019).

Invadopodia are structures found in cancer cells, which are "finger-like" membrane protrusions and exhibits proteolytic activity in peri-cellular matrix (Artym *et al.*, 2011; Murphy and Courtneidge, 2011; Hoshino, Branch and Weaver, 2013; Gould and Courtneidge, 2014). Besides actin, invadopodia has other key proteins to maintain the core, for instance cortactin, Tks 4 and 5, and MT1-MMP (Murphy and Courtneidge, 2011; Hoshino, Branch and Weaver, 2013; Saykali and El-Sibai, 2014). Cortactin and Tks regulate the actin cytoskeleton, remodeling the membrane to form the protrusions, and the address of MT1-MMP to the cell membrane, where it is crucial for peri-cellular

digestion and, consequently, tumor invasion (Weaver, 2008; Leong *et al.*, 2014; Eddy *et al.*, 2017).

Tumor cells' promotion to migrate and proliferate depends on the crosstalk with the ECM, and a main drive factor is hypoxia, since the aberrant growth of the tumor creates regions far from blood vessels and, consequently, with shortage of nutrient and oxygen. In these conditions, cells stabilize the Hypoxia-Inducible Factor 1-alpha (HIF1-α), a transcription factor sensitive to oxygen, which influences different aspects of tumor biology, leading to angiogenesis, invasion and metastasis (Gilkes, Semenza and Wirtz, 2014; Masoud and Li, 2015; Muz *et al.*, 2015; Semenza, 2016; Petrova *et al.*, 2018).

In this scenario, cells foster alterations in order to efficiently proliferate and invade; thus, proteins' regulations involved in cell growth, migration, cell survival, and metabolic changes are commonly found altered in tumors (Djagaeva and Doronkin, 2010; Hanahan and Weinberg, 2011; Sever and Brugge, 2015; Nazemi and Rainero, 2020).

Given the importance of hypoxia in tumorigenesis, here we extended our investigation on the role of peptide C16, derived from laminin, on tumor biology, mainly regulating invadopodia on fibrosarcoma (a malignant soft tissue cancer type) cells. It is noteworthy that our previous results were achieved with cells grown in normal oxygen tension situation, or normoxia. Hence, chemical hypoxia induced by cobalt (II) chloride ($CoCl_2$) and by exposing the cells to low tense of oxygen, allowed us to observe the hypoxic phenotype on HT1080 cells when also combined to peptide C16.

Furthermore, this study also delved into protein regulations, related to invadopodia formation and altered signaling pathways, by the peptide in conditions of normoxia and hypoxia. Moreover, we prompted to evaluate a possible remodeling mechanism driven by the peptide C16.

2. LITERATURE REVIEW

2.1 Fibrosarcoma

Fibrosarcoma is a malignant tumor derived from fibroblasts in soft tissues. According to a World Health Organization's description, fibrosarcoma histologically presents a "herringbone architecture" (Figure 2.1B), and its occurrence is rare. It was usually mistaken with other types of tumors, which an improved classification through the years helped to a better identification (Fletcher, Unni and Mertens, 2002; Folpe, 2014).

Sarcomas, or tumors from soft tissue, have no apparent cause, but as other types of cancers, their origin can relate to genetic factors, environmental aspects, irradiation, viral infections, and so on. There are reported cases of sarcoma derived from scar tissues, sites of fractures and also close to surgical implants (Hennipman and van Ginneken, 1985; Zindanci *et al.*, 2011; Fletcher CDM, Bridge JA, Hogendoorn PCW, 2013).

Since fibrosarcoma is derived from soft tissues, it can be found in different parts of the body, but mostly common around the neck, head, and trunk. Some studies have also detected fibrosarcoma in body extremities like in the foot, or within organs, such as uterus and ovaries; besides, it can also be found adjacent to bones (Blume *et al.*, 1997; Fletcher and Unni, 2002; Braun *et al.*, 2019; Miura *et al.*, 2019).

There is a slight difference between adult fibrosarcoma and the infantile one, despite both being histologically similar, the latter has a congenital cause (usually genetic) and has better prognosis, since the former one has high chances of promoting metastasis (Hajdu, 1998; Fletcher CDM, Bridge JA, Hogendoorn PCW, 2013).

Due to a variety of sarcoma tumors, fibrosarcomas used to mistakenly encompass other tumors, which increased the number of incidences reported. Nowadays, a more accurate diagnosis and techniques allows a better distinction, reducing the occurrences. According to a study from SEER (Surveillance, Epidemiology, and End Results) in 2006, the incidence of adult fibrosarcoma was 3.6% (National Institute for Health and Clinical Excellence, 2006). Usually, it can affect people in the late third decade of their life, but it is more common in older people, with an average of 30-60 years old (peak at 50 years

old). Apparently, there are no differences between sex; however, a few studies showed it can vaguely be more common in men (Toro *et al.*, 2006; Bahrami and Folpe, 2010; Fletcher CDM, Bridge JA, Hogendoorn PCW, 2013; Folpe, 2014).

In addition, due to its malignancy, fibrosarcoma cells usually metastasize, mostly to bones and lungs. Moreover, the tumor tends to be resistant to chemotherapy. Therefore, due to its difficulty detection (it is usually painless and commonly found incidentally when extracting unusual body masses for biopsy), and combined with a poor prognosis, the survival of the patients is around 50-70%, in 2-5 years (National Institute for Health and Clinical Excellence, 2006; Folpe, 2014).

In order to detect this cancer, a pathological analysis using basic histology and immunohistochemistry is necessary, and in some instances even electron microscopy. Nevertheless, other techniques have become crucial for a decisive diagnostic, such as x-rays, Magnetic Resonance Imaging (MRI), and Computed Tomography (CT). However, literature states the diagnosis is based on a process of exclusion from other tumors (Klijanienko and Lagacé, 2011; Franchi and Santucci, 2013; Augsburger *et al.*, 2017; Wang *et al.*, 2018).

Over the last years, attempts to identify specific characteristics from this tumor, led to a subclassification of fibrosarcomas: Low-grade Fibromyxoid Sarcoma, Sclerosing Epithelioid Fibrosarcoma, Acral Myxoinflammatory Fibroblastic Sarcoma and Epithelioid Myxofibrosarcoma. These variants are designated by their histomorphology, and traits observed by electron microscopy as well. The subtypes seem to be the reason why there is no consensus in particular cytogenetic alterations and other proteins, which could be used as markers for identification (Bahrami and Folpe, 2010; Fletcher CDM, Bridge JA, Hogendoorn PCW, 2013; Franchi and Santucci, 2013; Gibbs, Henderson-Jackson and Bui, 2016). Not surprisingly, the use of the general term “Fibrosarcoma” is on decline (Folpe, 2020).

Overall, fibrosarcomas have been related to gene and chromosomal abnormalities that lead to a poor prognostic, such as having numeric alterations and translocations. Besides, altered genes in fibrosarcomas correlates with genes that have roles in the

tumorigenesis in sarcomas (Limon *et al.*, 1998; Schmidt *et al.*, 2002; Klijanienko and Lagacé, 2011).

Fibrosarcoma cells originate from fibroblasts which are cells found in connective tissues (Figure 2.1A) and have an important role for producing the matrix extracellular components such as collagen type I, III, IV and V, fibronectin, and other molecules such as laminins. Furthermore, in regular tissues, fibroblasts commonly remodel these molecules, specially rearranging the collagen fibrils, but also degrading them when necessary, using enzymes, such as metalloproteinases (Kalluri and Zeisberg, 2006; Alberts *et al.*, 2007; Junqueira and Carneiro, 2013).

A **B**

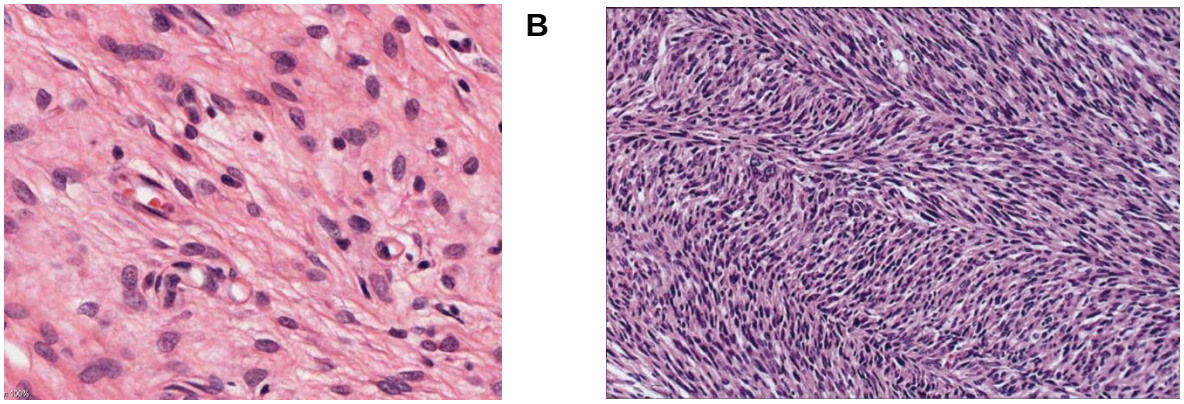

Figure 2.1: Comparison of connective tissue and the "herringbone architecture" of fibrosarcoma. (A) Histology micrograph from a (healthy) connective tissue, stained with H&E. (B) Histology micrograph from a fibrosarcoma tumor depicting the "herringbone architecture", stained with H&E, 140X (Hajdu, 1998).

Therefore, cancer cells derived from fibroblast also have the characteristic of producing collagen; even though it varies depending on the tumor subtype. The morphology of the tumor cells is usually spindle-like but can also appear as round shape. Microscopically observing, it is also common to detect multinucleated cells, and most variants of fibrosarcoma present abundant rough endoplasmic reticulum cisternae (Fletcher CDM, Bridge JA, Hogendoorn PCW, 2013; Franchi and Santucci, 2013; Folpe, 2020).

A cell type commonly used to study cancer and fibrosarcoma is the HT1080 cells. These cells were isolated in 1972 from a 35 year old man, who died without receiving chemotherapy or radiotherapy, and were characterized and established in 1974, as highly

proliferative and with lack of contact inhibition (Rasheed *et al.*, 1974). HT1080 cells are known to have the oncogene n-RAS activated, which can promote proliferation, cell differentiation and other disarrangement that may contribute to tumorigenesis (Hall *et al.*, 1983; Parker and Mattos, 2018).

2.2 Tumor Microenvironment

Cancers are not just a mass of cells that due to genetic alterations are able to continuously proliferate and control other aspects to maintain their status, such as avoid cell death. Tumor cells also depend on the environment surrounding them to get sustainment and to enhance the malignance in order to induce metastasis. This Tumor Microenvironment (TME) is composed of the Extracellular Matrix (ECM), and other cells and molecules that support cancer cells in their endeavour (Fingleton and Lynch, 2010; Balkwill, Capasso and Hagemann, 2012; Yang and Lin, 2017; Compton, 2021).

The non-cancer cells are recruited and reprogrammed by the tumor to enhance its abilities to promote the tumor growth and survival, remodeling of the ECM, migrate and invade adjacent sites. Many types of cells infiltrated the tumor, such as immune system cells, cancer-associated fibroblasts (CAFs), pericytes, adipocytes, cells from blood and lymphatic vessels nearby (Balkwill, Capasso and Hagemann, 2012; Hanahan and Coussens, 2012; Quail and Joyce, 2013; Anderson and Simon, 2020). To this day and age, diagnosis of a tumor prognosis is also counting the presence of TME elements, for example, the immune cells presented on it, which can determine a better and more specific treatment, such as immunotherapies (Kirkwood *et al.*, 2012; Junttila and De Sauvage, 2013; Riley *et al.*, 2019; Lee *et al.*, 2021).

Immune cells have a double-edged sword job inside the tumor microenvironment, as they can behave as anti- or pro-tumor. Cells from the innate immunity, such as macrophages, neutrophils, and dendritic cells, arrive at the tumor site in an attempt to establish the order. Secondary, adaptative immune cells, such as T and B cells, and natural killers, also directs to the tumor niche as responding to inflammation related to the tumor growth. However, if the immune cells are not succeeded to destroy the initial tumor,

it means that cancer cells were able to trick them by inducing cytokines to promote pro-tumoral development (Hinshaw and Shevde, 2019; Anderson and Simon, 2020; Labani-Motlagh, Ashja-Mahdavi and Loskog, 2020).

As for the stromal cells (fibroblasts, vascular endothelial cells, adipocytes, and others) within the tumor, the role is to secrete factors that influence all the tumor development stages, and also remodel the ECM. For instance, vascular endothelial cells as essential, maintain their tasks to provide nutrients, gas exchanges and other molecules to the tissues and ECM surrounding the blood vessels it consists, but also have great support on angiogenesis (creation of new blood vessels), and more surprisingly, can transform themselves to become CAFs. The CAFs then (originated by other types of cells, and obviously by original fibroblasts) have an important role in the TME by regulating factors and production and arrangement of the ECM. In particular, CAFs have an increased proliferation rate, thus it justifies the higher amount of ECM production. In addition, CAFs also produce and secrete specific and unique cytokines that help the tumor progression (Dvorak, 2015; Bussard *et al.*, 2016; Guan *et al.*, 2017; Denton, Roberts and Fearon, 2018).

Despite the cells that constitute the TME, a major component has also a greater role on the milieu: the ECM. Its importance may be auto explanatory, as it can be up to 60% of the tumor mass (Anderson and Simon, 2020). ECM is a dynamic network that combines different molecules and provides support and anchoring to cells, as well as exchange of components, nutrients, gases, and others. At the TME, ECM is fundamental to modulate the crosstalk between all the present cells and molecules and therefore induce tumor proliferation and development, migration, invasion and consequently, metastasis (Pickup, Mouw and Weaver, 2014; Kai, Drain and Weaver, 2019; Nazemi and Rainero, 2020).

Besides, the rearrangement of the ECM during the tumor growth is essential for tumor migration and then invasion (Kai, Drain and Weaver, 2019). It is remodeled by proteolytic enzymes, such as matrix metalloproteinases (MMPs) that cleaves the ECM molecules such as collagen, laminins and others (Egeblad and Werb, 2002; Lee and Murphy, 2004). This cleavage also releases bioactive fragments that studies have been

shown to also foster cancer malignance (Kikkawa *et al.*, 2013; Paz, Pathak and Yang, 2014; Kisling, Lust and Katwa, 2019). As part of the invasion process, cancer cells also induce invadopodia formation, a proteolytic protrusion that allows cells to move through the ECM after its degradation (Weaver, 2008; Eddy *et al.*, 2017).

Furthermore, as the tumor grows, some regions become poorly vascularized or even avascular, where nutrients and oxygen do not reach. These areas are then in hypoxia, or low tense of oxygen, which makes cancer cells stabilize a sensitive protein called Hypoxia-Inducible Factor 1- alpha, and as a transcription factor, it leads to activation of genes related to angiogenesis and other tumor progression traits (Gilkes, Semenza and Wirtz, 2014; Muz *et al.*, 2015; Unwith *et al.*, 2015; Petrova *et al.*, 2018)

ECM role in the TME and its composition, as well as its bioactive fragments generated by MMPs, the invadopodia formation and activity, and the hypoxic environment at the tumor niche, will be explored in more detail in the next topics.

2.2.1 Extracellular Matrix

The Extracellular Matrix (ECM) is an organized three-dimensional macromolecular and non-cellular network that lies between tissues and organs, serving as a scaffold to the cells. Although, the structure provides not just physical support to the cells, but also regulates a wide variety of cell behaviours, such as: cell morphology and polarity; signaling; proliferation (and growth); cell movements as adhesion, migration and invasion; survival; and differentiation (Daley, Peters and Larsen, 2008; Hynes, 2009; Theocharis *et al.*, 2016).

ECM is a dynamic structure where the production of its more than 300 components, are made by the cells around it, but can also be degraded when needed, creating a remodeling process (Alberts *et al.*, 2007; Lu *et al.*, 2011; Bonnans, Chou and Werb, 2014). Besides, in a mutual relationship of exchange components and information, ECM composition can alter the cells interior, and on the other hand, cells can alter the ECM depending on the situation. Thus, the biochemical and biomechanical properties of ECM regulates cell behaviour, and this crosstalk allows a homeostasis in the cells and

consequently, in the tissues (Frantz, Stewart and Weaver, 2010; Humphrey, Dufresne and Schwartz, 2014).

The main components of the ECM are water, proteins, and polysaccharides (Frantz, Stewart and Weaver, 2010; McKee *et al.*, 2019). Many authors group ECM components into fibrillar (or fibrous) proteins and non fibrillar components, even though some discrepancies are found between the classifications, mostly because the ECM elements connect with each other. In a nutshell, the fibrillar components are usually grouped by collagens, elastin, fibronectin and vitronectin. As for the non-fibrillar, are included: growth factors, as well as glycoproteins, such as laminins, proteoglycans (PGs), and glycosaminoglycans (GAGs). Some of these elements are very hydrated and acidic molecules (Daley, Peters and Larsen, 2008; Mouw, Ou and Weaver, 2014; Theocharis *et al.*, 2016; McKee *et al.*, 2019).

Most of these elements are big, and ECM proteins usually have folded domains, which may contain important information for biological behaviour in this environment (Schenk and Quaranta, 2003; Hynes, 2009). For instance, fibronectin, that participates on the ECM organization, and has a big role in cells attachment, can be extremely requested by the cells and that can uncover cryptic domains, which can signalling to changes in cell behaviour (Frantz, Stewart and Weaver, 2010; Xu and Mosher, 2011).

Furthermore, glycoproteins, in majority the laminins (as well as fibronectin, vitronectin, thrombospondin, tenascin, nidogen, nephronectin, fibrinogen, and others), are proteins that have small oligosaccharide chains, usually ramified. As for the glycosaminoglycans, which are linear and long polysaccharides chains that contain amino sugars (amino monosaccharides), are divided into two groups: sulfate group (chondroitin sulphate, heparan sulphate, dermatan sulphate and heparin sulphate) and non-sulphate in the group, that includes just the hyaluronic acid. Even more, the non-fibrillar components also have proteoglycans, which is a compound of many GAGs chains connected to a core protein, and have as examples: aggrecan, versican, neurocan, and brevican (Alberts *et al.*, 2007; Wight, Toole and Hascall, 2011; Xu and Mosher, 2011; Junqueira and Carneiro, 2013; Theocharis *et al.*, 2016; Balbinot-Alfaro *et al.*, 2021). Despite that, another important element is elastin fibers, which associate with collagen

fibers and provide stretch to regions that need more flexibility due to constant stretching forces (Muiznieks and Keeley, 2013; Theocharis *et al.*, 2016).

A major component found in ECM is collagen, consequently, it represents approximately 30% of all body protein, functioning as tensile strength as well as providing elasticity (Bosman and Stamenkovic, 2003; Muiznieks and Keeley, 2013). It is mainly produced by fibroblasts, which uses 42-46 genes to produce around 28 types of collagen, which the most common encountered are the types I, II, III, V and XI as fibrils, and type IV forming a net (Junqueira and Carneiro, 2013; Muiznieks and Keeley, 2013; Mouw, Ou and Weaver, 2014). As a macromolecule, after its multi-step production and assembly, it is modulated by other ECM components and rearranged by the fibroblasts and proteases, in other words, it is constantly remodeled (Frantz, Stewart and Weaver, 2010; Birk and Brückner, 2011; Engel and Chiquet, 2011; Mouw, Ou and Weaver, 2014).

Despite that, in cancer, a great deposition of collagen is commonly found on the tumor microenvironment ECM, receiving the name of desmoplasia, and it is related to poor prognosis (Geiger and Yamada, 2011; Anderson and Simon, 2020). For instance, Barry-Hamilton et al. (2010) demonstrated that lysyl oxidases (LOX), enzymes that promotes the cross-linking of the fibrils of collagen I on the ECM, are commonly found enhanced on the TME, thus, can be inhibited and used as target for therapeutic purposes (Barry-Hamilton *et al.*, 2010).

Therefore, the remodeling of the ECM highlights the dynamic process of this structure. Cells use different kinds of proteases, especially matrix metalloproteinases to degrade ECM elements such as collagens, laminins, fibronectin and others (Bonnans, Chou and Werb, 2014). For instance, it has been reported that collagen and laminin derived fragments from MMPs cleavage can act as pro- or anti- diseases (Kikkawa *et al.*, 2013; Kisling, Lust and Katwa, 2019).

In particular, in tumors, ECM promotes the induction of metastasis by a combination of factors that in general consists of inducing desmoplasia, and abnormal increase of several ECM components, which also modulates the stiffness (Kai, Drain and Weaver, 2019). Also, influence from the CAFs contribute to enhancing the phenomena and inducing cancer cells to change to an epithelial-mesenchymal transition (EMT).

Following that, together (and driven by TME forces, like hypoxia), ECM and cancer cells dynamically and aberrantly enhances MMPs and other proteases secretion, that would degrade ECM components to allow the cancer cells to migrate and invade through the surroundings (Bonnans, Chou and Werb, 2014; Kaushik *et al.*, 2019; Henke, Nandigama and Ergün, 2020).

2.2.1.1 Basement Membrane

The Basement Membrane (BM) is no more than a specialized region of the ECM. It resides mostly under epithelial cells and endothelial cells, connecting these cells to the adjacent tissues, like the connective tissue, or even to the major ECM (Miner, 2011; Jayadev and Sherwood, 2017).

In 1840, William Bowman observed a delicate membrane in muscle tissue and later designated it as “basement membrane” (Bowman, 1840; Ashton, 1974; Yurchenco, 2011). Following to that, evidence of a thin layer in other tissues started to be observed by some microscopists, being better visualized if tissues are stained with Periodic acid-Schiff (PAS) reagent (Ashton, 1974; Miner, 2011; Yurchenco, 2011). However, it becomes evident when observed by Electron Microscopy (EM), since it appears as an electron-dense and thin layer that can range between 40 to 120 nm of thickness. Even though, studies also shown that differences of the arrangement and protein distribution creates sublayers (or laminae) when further analysed by EM, which generates some divergence on literature, as some authors consider the subclassification as “basal lamina” (or may distinguish as lamina densa and/or lucida and fibroreticularis), and others categorize just as BM (Bosman and Stamenkovic, 2003; Kalluri, 2003; Alberts *et al.*, 2007; Yurchenco, 2011; Mak and Mei, 2017).

The composition of the BM consists mainly in laminins, collagen IV, perlecan and nidogen (entactin). More specifically, laminin and the collagen IV, as the main core proteins, are assembled in networks, which are then connected by other ECM molecules, mainly perlecan and nidogen. Just collagen IV alone makes up to 50% of all BM. And the main function of this structure is to anchor the cells and give them structural support

(Kalluri, 2003; LeBleu, MacDonald and Kalluri, 2007; Jayadev and Sherwood, 2017; Mak and Mei, 2017).

As like the major ECM, cells can also cleave the molecules at the BM by MMPs, turning them into fragments and peptides, which may have some effect on the cells, as some cryptic domains, for example, in laminins, can become available. In cancer, an increasing number of studies has been showing that those peptides may increase some tumor traits (Kalluri, 2003; Schenk and Quaranta, 2003; Kisling, Lust and Katwa, 2019). In particularly, this phenomenon occurs mostly in invadopodia (tumor protrusions that triggers MMPs) sites, in attempt of cells to migrate and invade (Wells, Gaggar and Blalock, 2015; Horejs, 2016; Kai, Drain and Weaver, 2019; Iizuka *et al.*, 2020).

2.2.1.2 Laminin

Laminins are trimeric cruciform shaped glycoproteins presented on the ECM, particularly at BM regions. It was discovered around 40 years ago, concomitant in 2 laboratories, as a glycoprotein encountered in the ECM produced by mouse tumor cells; also, antibodies against this glycoprotein reacted to the BM in mouse tissues. Therefore, the name laminin was given since BM is also known as basal laminae (Aumailley, 2013; Hohenester, 2019).

These adhesive glycoproteins have a large molecular weight varying from 400 to 900kDa. Besides, it generates networks together with other molecules at the BM and are essential anchors for the cells in the adjacent tissue, such as epithelial and endothelial cells. In fact, cells bind to laminin by receptors at the cellular membrane, such as by integrins (a transmembrane - heterodimer with subunits α and β - receptor) (Aumailley, 2013; Hohenester and Yurchenco, 2013).

Laminin, as a heterotrimeric glycoprotein, is formed by 3 disulfide-linked polypeptides subunits: α, β, and γ chains. Isoforms of laminin presents different combinations from the 3 chain subunits, which consists in five of the α, four of the β and three of the γ chains, each variant being encoded by one of the 11 genes associated.

Alternative splicing can also occur to make different variants (LeBleu, MacDonald and Kalluri, 2007; Yao, 2017).

The 16 laminins isoforms found so far are named after their chain composition. In the past, they received their names according to their discovery. However, due to many possibilities of the variant chain arrangements, which can give around 60 potential molecules of laminin, the chain subunits now designate the specific laminin. For instance, the isoform Laminin-111 (named before as Laminin 1) received this name due to the subunits being α1, β1 and γ1 (Aumailley *et al.*, 2005; Hohenester, 2019; de Siqueira, Freitas and Jaeger, 2022).

Table 2.1. Laminin isoforms and chain composition

Laminin isoform	Subunits chains
Laminin 111	α1, β1, γ1
Laminin 121	α1, β2, γ1
Laminin 211	α2, β1, γ1
Laminin 213	α2, β1, γ3
Laminin 221	α2, β2, γ1
Laminin 311	α3, β1, γ1
Laminin 312	α3, β1, γ2
Laminin 321	α3, β2, γ1
Laminin 322	α3, β2, γ2
Laminin 411	α4, β1, γ1
Laminin 421	α4, β2, γ1
Laminin 422	α4, β2, γ2
Laminin 423	α4, β2, γ3
Laminin 511	α5, β1, γ1
Laminin 521	α5, β2, γ1
Laminin 523	α5, β2, γ3

Besides that, the isoforms seem to be cell and tissue specific distributed. For instance, at the endothelial BMs, there are plenty of laminins 411 and 421. Laminin-111 is ubiquitously located during embryonic stages, but in adults resides just in small amounts in some BMs. On the other hand, isoforms that contain α5 subunit (like laminins 511 and 521) are more common in adults (Miner *et al.*, 1997; Aumailley, 2013).

The structure made by the 3 chains composes a laminin shape into a 4-arm shape, in which 3 of the arms are composed of each chain separately, and the other arm, also called the long arm, is an alpha-helical coiled-coil that entwines the 3 chains. Also, through this structure, specific domains can be found, such as: globular domains (LG), laminin N-terminal (LN) domains, Epidermal Growth Factor-like (LE) domains, and laminin type IV (L4 and LF- globular) domains. Some globular domains are localized by the end of the long arm, and are separated into 5 domains (LG1, LG2, LG3, LG4 and LG5). The other globular domains, L4 and LF, as well as LN and LE domains are spread through the 3 short arms (Aumailley, 2013; Hohenester and Yurchenco, 2013; Hohenester, 2019).

These motifs are specific to allow interaction with cells or other molecules, as well for a self assembly at the BM. For instance, the N-terminal LN domain promotes the binding to integrins α1β1 and α2β1. As for the LE domains, it can bind to nidogen and through that modulate the interaction with collagen IV and perlecan, assembling the network at the BM (Miner and Yurchenco, 2004; Borycki, 2013; Yao, 2017).

Despite the adhesive and structural functions, laminins also play a role in the ECM crosstalk with cells, by signaling when binding to cell receptors and in migration during embryogenesis or wound healing process. Interestingly, experiments testing the lack of laminin demonstrated that BM network was poorly assembled, or a lost of function from the components from BM (collagen IV, perlecan and nidogen) was noted (Colognato and Yurchenco, 2000; Mak and Mei, 2017).

Over the last decades, in order to further understand laminin functions, the fragmentation of laminin, which can also occur *in vivo* by MMPs action, has been helping to overcome its traits on the ECM and on diseases. Consequently, some peptides derived from laminin, which are binding sites, have been identified to induce not just cell adhesion, but also migration, as well as inducing cell behaviour towards tumorigenesis, such as invasion, angiogenesis, tumor growth, among others (Nomizu *et al.*, 1997; Freitas *et al.*, 2007; Kikkawa *et al.*, 2013; Siqueira *et al.*, 2016; Smuczek, 2019). More characteristics and effects from peptides derived from laminin will be elucidated on the next topic.

2.2.1.3 Peptides derived from Laminin

As like the other ECM molecules, laminin can be cleaved by proteases such as MMPs. The cleavage results in fragments or peptides, that have been shown to affect the cells and induce some effect, that can be pro- or anti- diseases. More specifically, when the fragmentation occurs, cryptic sites get exposed opening a wide range of possibilities and effects in cell behaviour. In particular for the ECM molecules, the fragments from these hidden regions (matricryptic sites) can also be called as “matrikines” (Schenk and Quaranta, 2003; Mott and Werb, 2004; Wells, Gaggar and Blalock, 2015; Horejs, 2016).

To understand the function and effects of domains and sites from laminin, fragments cleaved by proteolysis and synthetic peptides have been used. Hence, Nomizu’s group has been screening all laminin isoforms through nearly 30 years, and produced more than 3000 synthetic peptides that correspond to all laminin’s sequences (Negishi and Nomizu, 2019). According to that, peptides from laminin-111, has been mapped out and its binding sites revealed, allowing 673 peptides to be synthetized (Nomizu *et al.*, 1995, 1997, 1998; Kikkawa *et al.*, 2013).

As the most studied laminin, due to its commercially availability and possibility of isolation from the high amounts available at a mouse tumor (Engelbreth-Holm-Swarm - EHS), the laminin-111, which bind to cells, BMs molecules and to itself, has also been related to a tumorigenesis tool. Studies *in vitro* and *in vivo*, showed that laminin-111 is commonly found in tumors, and can induce a malignant phenotype on cells, as it promotes cell adhesion, migration, tumor growth, release of proteases (such as the MMPs 2 and 9) and metastasis (Faisal Khan and Falcone, 1997; Engbring and Kleinman, 2003; Kikkawa *et al.*, 2013; Horejs *et al.*, 2014).

Therefore, the effects from laminin-111 have been uncovered with the help of its synthetic sequences, which usually have around 6-12 amino acids. Moreover, these bioactive peptides can trigger effects as: cell protection against malignancy, migration (in wound healing and tumor), invasion, angiogenesis, secretion of proteases, invadopodia formation, alteration in signaling pathways, tumor growth, metastasis, and others (Ponce, Nomizu and Kleinman, 2001; Kuratomi *et al.*, 2002; Engbring and Kleinman, 2003; Freitas

et al., 2004; Nascimento *et al.*, 2010; Siqueira *et al.*, 2016; Smuczek, 2019; Caires-dos-Santos *et al.*, 2020; de Siqueira, Freitas and Jaeger, 2022). From several bioactive peptides that show important roles, some have been further investigated, for instance: YIGSR, IKVAV, AG73, C16 (Figure 2.2).

The YIGSR, derived from the β1 chain, was the first laminin-111 peptide described and is well-studied. Despite promoting cell adhesion and migration, YIGSR is known by its attribute to inhibit the malignancy, by blocking angiogenesis and metastasis, which makes it a good candidate for therapy development (Graf et al., 1987; Iwamoto, Yukihide et al., 1987; Sakamoto et al., 1991; Yoshida et al., 1999; Engbring and Kleinman, 2003). Therefore, new studies have been using this short peptide associated with liposomes, micelles, and nanoparticles to deliver it to tumors as a therapeutic attempt (Dubey, Singodia and Vyas, 2010; Ukawala *et al.*, 2012; Carvalho *et al.*, 2019).

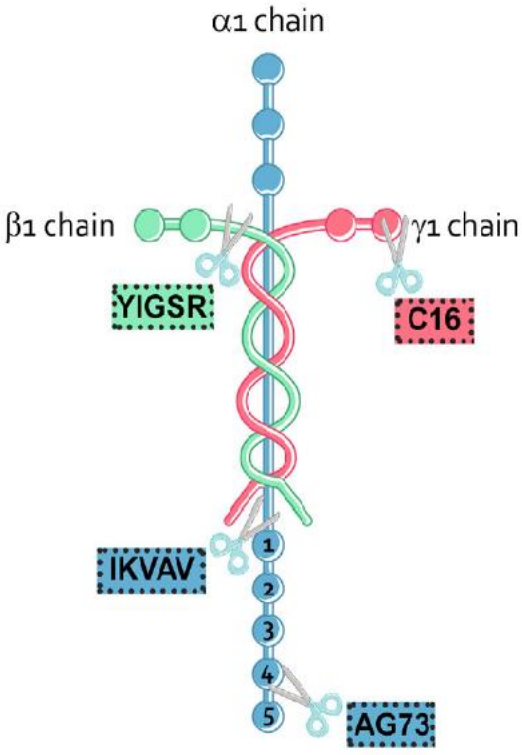

Figure 2.2: Laminin-111 and its main bioactive peptides. Schematic model of Laminin-111, depicting the sites of protease cleavage and release of the peptides YGSR at the β1 chain, C16 at the γ1 chain, IKVAV at the α1 chain, and AG73, at the globular domain (LG4) of the α1 chain. Adapted from de Siqueira, Freitas and Jaeger, 2022.

On the other hand, the other peptides are usually associated with tumor progression. Thus, as for the IKVAV (or SIKVAV), from the α1 chain, the induction of adhesion, migration, tumor growth, angiogenesis and metastasis were observed (Tashiro *et al.*, 1989; Kanemoto *et al.*, 1990; Grant *et al.*, 1992; Nomizu *et al.*, 1992). Moreover, studies from our group demonstrated that IKVAV plays a role in MMPs secretion, which was regulated by integrins mediated through ERK1/2 pathway, in human adenoid cystic carcinoma cells (Freitas *et al.*, 2004, 2007; de Siqueira, Freitas and Jaeger, 2022).

Furthermore, the AG73 (RKRLQVQLSIRT), localized at the globular domain on the α1 chain, has been related to adhesion, migration, invasion, and protease secretion (MMPs), and invadopodia formation and activity (Nomizu *et al.*, 1995; Gama-de-Souza *et al.*, 2008; Siqueira *et al.*, 2010; Nascimento *et al.*, 2011). Some of these effects were demonstrated by our group, that also observed the AG73 affinity to syndecan-1 and integrin β1 receptors, in human adenoid cystic carcinoma cells (Nascimento *et al.*, 2011). Similar findings were also demonstrated by other studies, showing that AG73 indeed seems to bind to syndecans, a receptor that is enhanced in tumors (Kikkawa *et al.*, 2013; Puchalapalli *et al.*, 2019; Czarnowski, 2021). Hence, a new therapy mediating genes delivery through AG73 binding to syndecan-2, may be a promising gene therapy for cancer (Negishi and Nomizu, 2019).

2.2.1.4 Peptide C16

As the objective of this study, peptide C16 (KAFDITYVRLKF), localized at the first globular domain at the γ1-chain of laminin-111 has been shown to induce adhesion, migration, proteolytic activity and invadopodia formation and activity (Kuratomi *et al.*, 2002; Pinheiro *et al.*, 2011; Kikkawa *et al.*, 2013; Siqueira *et al.*, 2016; Smuczek, 2019; de Siqueira, Freitas and Jaeger, 2022).

In particular, since its first synthesis, peptide C16 demonstrated its powerful adhesion properties when human fibrosarcoma cells (HT1080) and mouse melanoma cells (B16F10) highly attached to it and also easily spread after, in comparison to other peptides and controls (Nomizu *et al.*, 1997). In addition, peptide C16 sites may play an

important role in the γ1-chain of laminin for endothelial cells to bind, and then foster angiogenic properties (Ponce *et al.*, 1999; Ponce and Kleinman, 2003). Also, peptide C16 enhanced migration in mouse and human melanoma cells, as well as lung metastasis *in vivo*, which was also associated with increase of MMP-9 (Kuratomi *et al.*, 2002; Lugassy *et al.*, 2007).

Moreover, evidence suggests peptide C16 binds to the integrins αvβ3 and α5β1 (Ponce, Nomizu and Kleinman, 2001). And our group demonstrated an increase in phosphorylation of ERK 1/2 and Src after the peptide connection to the integrin subunit β1, in fibrosarcoma, and squamous cell carcinoma cells; and this adhesion was decreased when a β1 knockdown was performed, which was also observed in breast cancer cells (Siqueira *et al.*, 2016; Smuczek *et al.*, 2017; Galheigo *et al.*, unpublished).

In other results from our group, the cleavage of laminin-111 was observed in tumor samples and in breast cancer cells, by the action of MMPs, and proteomic analysis detected the peptide C16 among the fragments (Smuczek, 2019). Despite that, peptide C16 seems to induce GPNMB, a gene related to enhancing a malignant phenotype in breast cancer cells (Smuczek *et al.*, 2017).

Interestingly, peptide C16 induced invadopodia formation and activity in adenoid cystic carcinoma cells (CAC2), fibrosarcoma cells (HT1080), and prostate cancer cells (DU145). In fact, the integrin β1 subunit and its inducing signalling through ERK1/2 and Src correlated with increase of invadopodia activity. Besides, an increase in reactive species of oxygen (ROS) concurrent with invadopodia key-proteins, as Tks, cortactin and MT1-MMP (Nascimento *et al.*, 2011; Siqueira *et al.*, 2016; Caires-dos-Santos *et al.*, 2020).

More recent, peptide C16 was depicted in vesicles inside breast cancer cells when observed by EM and immunofluorescence, and further investigation leaded to find the peptide associated to caveolin-1 vesicles, early endosomes, and additionally in lysosomes (Galheigo *et al.*, unpublished).

2.2.2 Matrix Metalloproteinases

The matrix metalloproteinases (MMPs), also known by “matrixins”, are proteases that remodel and maintain the ECM. These zinc-dependent endopeptidases can degrade all components presented on BM and ECM. By doing so, MMPs play different roles at the ECM and on the cells on it, as in proliferation, migration, differentiation, and are also involved in embryogenesis, apoptosis, angiogenesis, tissue repair, immune response and others (Lee and Murphy, 2004; Löffek, Schilling and Franzke, 2011; Cui, Hu and Khalil, 2017; Cabral-Pacheco *et al.*, 2020).

MMPs perform the cleavage of the ECM molecules, which not just promotes a remodeling process on the environment, but also releases fragments, or matrikines, that could be bioactive and induce signals to cells to alter their behaviour, as well as foster changes in the ECM composition. Likewise, this process also releases growth factors, cytokines and chemokines arrested at the ECM (Lee and Murphy, 2004; Mott and Werb, 2004; Wells, Gaggar and Blalock, 2015).

To date, 28 MMPs are known in vertebrates, from that, 24 are found in human, which 14 are usually detected in vasculature (Visse and Nagase, 2003; Lee and Murphy, 2004; Cui, Hu and Khalil, 2017; Laronha and Caldeira, 2020). The MMPs belong to a multidomain zinc-dependent superfamily of proteases, called metzincins, which also aggregate the proteases ADAMs (proteins that have a disintegrin and metalloprotease domain) and ADAM-TS (ADAM with a thrombospondin-like motif) (Mott and Werb, 2004; Laronha and Caldeira, 2020). MMPs are classified according to the substrate it cleaves and to the organization of these proteins structure domains, as: collagenases, gelatinases, stromelysins, matrilysins, membrane-type metalloproteinases (MT-MMPs), and other metalloproteinases (Visse and Nagase, 2003; Nagase, Visse and Murphy, 2006; Cui, Hu and Khalil, 2017).

All MMPs have the same core, which consists in 4 domains: a propeptide (N-terminal prodomain), a catalytic domain (containing the zinc binding motif), a linker peptide (hinge region), and a hemopexin (C-terminal hemopexin-like) domain. Then, according to their function or location, the MMPs types may have reduction or additional domains or

motifs. For instance, the MT-MMPs have an extra domain, by which they bind to the cell membrane; the gelatinases MMP-2 and MMP-9 have 3 repeated motifs of fibronectin type II-like, and MMP-7 and MMP-26, also called “minimal MMPs”, lack the linker peptide and the hemopexin domain (Itoh, 2015; Stawikowski and Fields, 2015; Cui, Hu and Khalil, 2017).

In summary, the regulation of MMPs activation involves 4 stages: 1- gene expression (including transcriptional and post-transcriptional regulation); 2- secretion (also called compartmentalization); 3- activation of the proenzyme (pro-MMP or zymogen, by removal of the pro-domain) to active enzyme; 4- presence of specific inhibitors (TIMPs). The activation process (at the third stage) is usually mediated by other proteases, as well as other MMPs types. Despite that, some variances occur depending on the MMP type, for instance, MT-MMPs are first intracellular activated to then proceed to the cytoplasmic membrane, where it can promote the cleavage or activate other MMPs (Kessenbrock, Plaks and Werb, 2010; Löffek, Schilling and Franzke, 2011; Cui, Hu and Khalil, 2017).

In regular tissues, MMPs are produced and activated in a balanced way, according to the environment requisition. As ECM molecules get produced, they need to be arranged or assembled, so MMPs cleave certain regions at those molecules, allowing cells to model them at the milieu. Moreover, in wound healing process and diseases, like cancer, MMPs permit cells to start migrating and invading through the ECM, after the site’s degradation (Löffek, Schilling and Franzke, 2011; Manou *et al.*, 2019).

Nevertheless, this process is also controlled by the MMPs regulators, the TIMPs, in other words, the tissue inhibitor of MMPs. This regulation is what modulates the homeostasis in healthy tissues. Therefore, in tumors, the MMP-TIMP balance is disrupted, provoking instability, which further permits cancer cells to migrate, invade and consequently, lead to metastasis. Hence, MMPs are being used as biomarkers to detect some types of cancer, and there is an aspiration in developing therapies targeting these proteases (Kessenbrock, Plaks and Werb, 2010; Cabral-Pacheco *et al.*, 2020).

Several MMPs participate in tumorigenesis, and according to their roles, studies are trying to uncover tumor traits through its process. For instance, enhanced levels of the gelatinases MMP-2 and MMP-9, and the MT1-MMP are correlated with a tumor malignant

phenotype, as they are related to increased proliferation, migration, invasion, angiogenesis, and metastasis.

Briefly, MMP-2, is ubiquitous to all cells, and cleaves collagen at the cell pericellular region, after being activated by MT-MMPS, like MT1-MMP. In addition, MMP-2 can bind to some receptors, like integrin and syndecans, mediating signalling through them, and furthermore degrade them. As for the MMP-9, the gelatinase is produced by many kinds of cells, such as neutrophils, macrophages, fibroblasts, epithelial cells, among others. Moreover, expression of MMP-9 can be triggered by inflammation, hypoxia and stimulus from growth factors, cytokines, and chemokines. MMP-9 can cleave a variety of ECM components, such as laminin, collagen IV and V, elastin, fibronectin, and proteoglycans. And finally, the membrane-type 1 – matrix metalloproteinase (MT1-MMP), also known as MMP-14, is a transmembrane protease, that is commonly associated with invadopodia activity, and promotes collagen (mostly fibrillar collagen, like collagen I) degradation. Besides, it is also associated with regulation of signaling pathways (Egeblad and Werb, 2002; Itoh, 2015; Cui, Hu and Khalil, 2017; Henriet and Emonard, 2019; Barillari, 2020).

2.2.3 Invadopodia

Invadosomes are structures with proteolytic activity that promote remodeling of ECM, which allow cells to further invade through the degraded area. In this category, podosomes and invadopodia are included (Linder, Wiesner and Himmel, 2011; Murphy and Courtneidge, 2011; Seano and Primo, 2015). Podosomes are formed by some types of cells that need to cross tissue barriers, such as monocytes and macrophages, or by osteoclasts that perform constant remodeling on their milieu. Besides, podosomes are also found in diseases that go through remodeling process of the ECM, for instance, atherosclerosis and aortic aneurysms. On the other hand, invadopodia are the invadosomes that drive cancer towards metastasis (Weaver, 2008; Linder, Wiesner and Himmel, 2011).

Other protrusive structures related to cell motility, such as lamellipodia and filopodia, extension projections of actin-cytoskeleton and cytoplasmic protrusions,

respectively, also play a role in diseases, like cancer, however, they do not possess proteolytic features, as the invadosomes (Machesky, 2008; Saykali and El-Sibai, 2014; Om Alblazi and Siar, 2015).

Invadosomes were discovered in 1980 by David-Pfeuty and Singer, when observing chicken embryo fibroblasts cultured that was transformed by Rous Sarcoma Virus (RSV), and noted circular structures on the cells, which they called “rosettes” and were formed of vinculin and α-actinin (from focal adhesions) (David-Pfeuty and Singer, 1980). Later, other investigations demonstrated that those structures were located at the ventral membrane, in a shape like feet on the cell, which was called podosomes. Chen renamed them to invadopodia, or “invasive-feet”, in 1989, when he noted they had degradative ability (Chen, 1989). Since then, many studies have been trying to uncover invadopodia components and functions (Murphy and Courtneidge, 2011; Augoff, Hryniewicz-Jankowska and Tabola, 2020; Revach, Grosheva and Geiger, 2020).

In detail, invadopodia are invasive finger-like structures, or protrusions rich in actin, with proteolytic activity to degrade the ECM (Weaver, 2008; Saykali and El-Sibai, 2014). These dynamic structures are only observed in cancer cells, and can be considered a hallmark for a malignant phenotype, since cells that present it have acquired enough traits to perform degradation of its surroundings (ECM and tissues barriers), and therefore can extravasate to vasculature and disseminate, leading to metastasis (Eddy *et al.*, 2017).

Initially, invadopodia was mainly observed *in vitro*, at 2D cell lines assays that degrade (fluorescent) substrates, but more recently, studies in mouse and chicken models, and specially assessment in human cancer patients, confirmed the presence of the structure *in vivo* (Artym, Yamada and Mueller, 2009; Bergman, Condeelis and Gligorijevic, 2014; Leong *et al.*, 2014).

Despite the fact that invadopodia shares some similarities with podosomes, like the main assembled proteins and the actin-core, they also differ in size, as invadopodia tend to be longer protrusions. In addition, a couple of studies have been demonstrating not just a spatial difference but also in time, as podosomes can be formed and disassembled in minutes, but invadopodia may take hours through all its processes (Weaver, 2008; Linder, Wiesner and Himmel, 2011; Murphy and Courtneidge, 2011).

ECM molecules and signals, as well as conditions performed by associated tumor cells, like release of growth factors (e.g., EGF, PDGF and TGFβ), hypoxia and low pH (acidic milieu), seems to trigger invadopodia formation (Hoshino, Branch and Weaver, 2013; Gould and Courtneidge, 2014; Masi *et al.*, 2020). Its structure is formed by a wide number of scaffolding and adhesive proteins to assemble the actin-core. Some key-proteins involved in this process are: cortactin, neural Wiskott-Aldrich syndrome protein (N-WASP), adaptor proteins as Tyrosine kinase substrate with four SH3 domains (Tks4) and Tyrosine kinase substrate with five SH3 domains (Tks5), Src and MT1-MMP (Weaver, 2008; Murphy and Courtneidge, 2011; Cmoch, Groves and Pikuła, 2014) .

Over the past years, some reviews are combining invadopodia formation studies, and are classifying the structure assembly into 3 main stages: 1- invadopodia precursor core initiation; 2- invadopodia precursor formation; 3- invadopodia maturation/stabilization (Murphy and Courtneidge, 2011; Saykali and El-Sibai, 2014; Eddy *et al.*, 2017).

Briefly, when the cell receives the stimuli from the ECM (or other cells), for instance, by growth factors, the first stage begins through a primary regulation of focal adhesions (FA) to the ECM, modulated by integrins, Src and FAK. Even though this process is not entirely elucidated and some studies diverge, it seems that a downstream of FAK occurs (this kinase is usually overexpressed in cancer), releasing Src, which then binds to Tks5, phosphorylating it (p-Tks5). Thus, p-Tks5 directs to regions with the phospholipid Phosphatidylinositol-3,4,bisphophate (Ptdlns(3,4)P_2) at the cell membrane, where it will colocalize to cortactin precursors and recruit cortactin (Cmoch, Groves and Pikuła, 2014; Saykali and El-Sibai, 2014; Revach, Grosheva and Geiger, 2020).

At the second stage, the formation of the structure *per se*, starts by recruitment of an actin regulatory complex: ARP2/3 binding to WIP (WASP-interacting protein). Besides, some key proteins get phosphorylated, such as cortactin, Tks5, fascin, AFAP110 (110kDa actin filament-associated protein). This step also led to ROS production, which is thought to be related to Tks 4 and 5, and the release of MMPs. Following that, Tks5 binds to actin regulators, as: NCK1, NCK2, Grb2 and N-WASP. Cortactin then associates with N-WASP and ARP2/3 complex, generating a WIP- dynamin complex (Murphy and Courtneidge, 2011; Saykali and El-Sibai, 2014).

The regulation of this assembly is mainly maintained by cortactin and Tks5, which leads to the next stage: maturation. Moreover, cortactin regulates the actin polymerization and assembly, by also inhibiting cofilin, a protein that depolymerizes actin. Despite that, cortactin also plays a role in triggering MMPs, and then modulates the secretion and localization of MMP-2, MMP-9 and MT1-MMP (Clark *et al.*, 2007; Jeannot and Besson, 2020).

In particular, MMP-2 and MMP-9 are secreted outside of the cell at the pericellular region, whereas MT1-MMP is inserted at the cell membrane, usually at the tip of the invadopodium (Egeblad and Werb, 2002; Weaver, 2006; Clark *et al.*, 2007). Besides, integrins are also known to appear at the invadopodium site, as they can trigger signals for cytoskeleton organization. Integrins subunits β1 and β3 have been associated with invadopodia, however little is known, and a few studies reports of integrins appearing at the site, like α5β3, α3β1, α6β1 and αvβ5 (Deryugina *et al.*, 2001; Weaver, 2006; Peláez *et al.*, 2017).

Moreover, the turnover and dissolution of the assembly is not well comprehended, but it is believed that phosphorylation of the key-proteins plays a part in slowly dissolving the core structure (Saykali and El-Sibai, 2014).

2.2.4 Hypoxic Tumor Microenvironment

In the TME, the altered cancer cells use two of its hallmarks - enhanced proliferation and evasion of apoptosis – to promote the tumor growth (Hanahan and Weinberg, 2011). The tumor mass, consisted of the tumor cells, the tumor associated cells and the ECM, that grows to an extend where regions of this niche become far from vessels, thus having restricted access to oxygen and nutrients. Some of these areas, can have a complete shortage of oxygen, and is not unusual to observe areas with necrosis inside tumors, where cells do not survive (Gilkes, Semenza and Wirtz, 2014; Petrova *et al.*, 2018).

Although, in regions with low availability of oxygen (hypoxia regions), cancer cells adapt to overcome this situation, by sensing the environment and stabilizing a

transcription factor, which in regular oxygen conditions gets hydroxylated, what allows it to bind to a tumour suppressor protein (von Hippel-Lindau protein, pVHL) that leads the factor to ubiquitination and then proteasomal degradation. The Hypoxia-Inducible factor-1 (HIF1) belongs to a family of transcription factors (HIF1, HIF2 and HIF3) that can sense the availability of oxygen. HIF1 is a heterodimeric protein that consists of subunits α (oxygen-destructible) and β (oxygen-indestructible). It is at the subunit α where the protein gets hydroxylated and then leads to degradation, by using O_2 as a substrate during the hydroxylation. Therefore, when oxygen is not available, the reaction is not processed, and HIF1-α remains intact (Brihimi-Horn and Pouysségur, 2009; Gregg L. Semenza, 2010b).

Following that, HIF1-α translocates to the nuclei where it makes a complex with HIF1-β and can promote the transcription of a wide number of genes. For instance, during hypoxia around 1-1.5% of the genome is transcript, as studies have been related HIF1-α to hundreds of genes (Gregg L Semenza, 2010; Ajdukovic, 2016; Pezzuto and Carico, 2018). Moreover, a database predicted that HIF1-α can target 2450 genes (*Gene Set - HIF1A*, 2021).

Therefore, HIF1-α when activated by hypoxia or stress conditions can regulate genes that will support the cells to face and adapt to the harmful environment. For instance, it has been reported that HIF1-α modulate genes associated to all aspects in cancer, such as: angiogenesis, reprogramming of metabolism, cell proliferation, remodeling of the ECM, epithelial-mesenchymal transition (EMT), cell motility (i.e., adhesion, migration and invasion), metastasis, cancer stem cells maintenance and resistant traits as immune evasion and resistance to chemotherapy and radiation therapy (Semenza, 2013; Schito and Semenza, 2016; Pezzuto and Carico, 2018).

In a nutshell, by reprogramming the cells to adapt to the hypoxic environment, HIF1-α promotes formation of new blood vessels by activating genes related to angiogenesis (e.g., EPO, VEGF), thus allowing the arrival of blood and consequently oxygen and nutrients to the cancer cells. In addition, in the TME, the cancer cells, by acquiring invasive characteristics that can also being influenced by hypoxia, induce invadopodia formation and degradation of the ECM and with the help of the associated tumor cells, will migrate through the tumor mass towards these new vessels, then

intravasate. On the vessels, cells travel through the blood flow, until reach a premetastatic niche, by extravasating the vessels and then repopulate the niche (Quail and Joyce, 2013; Muz *et al.*, 2015; Petrova *et al.*, 2018; Masi *et al.*, 2020).

Thus, since hypoxia plays an important role in tumorigenesis and can be a driven force that leads to metastasis, and also due to the fact that hypoxia promotes resistance of the tumor to chemotherapy and radiotherapy, therapeutic approaches to hypoxia are a viable pathway to target cancer, such as using HIF inhibitors (Semenza, 2003; Gregg L. Semenza, 2010a; Wilson and Hay, 2011).

2.3 Cancer cellular stress mechanisms

Cancer cells are constantly adapting to overcome the instabilities and inhospitable circumstances the TME imposes. For instance, tumor cells must face hostile conditions like hypoxia, nutrient deprivation, low pH, inflammation, DNA damage, oxidative stress, and high metabolic demand. Thus, if cells want to survive, they need to dynamically adjust the intracellular responses (Leprivier *et al.*, 2015; Cubillos-Ruiz, Bettigole and Glimcher, 2017; El-Naggar and Sorensen, 2018).

The mechanisms by which cells undergo to avoid cell death in the harmful conditions, include adaptations in protein regulations, such as protein synthesis as well as protein modulations (Clemens and Bommer, 1999; Sriram, Bohlen and Teleman, 2018). The regulation of protein translation occurs mostly in ribosomes, located on the cytoplasm or in the Endoplasmic Reticulum (ER). As for the protein modulations, including protein folding, translocation, and post-translational modification, are also mainly performed at the ER and sometimes can also be modified (e.g., receiving polysaccharides chains, like in GAGs) at the Golgi Apparatus (Alberts *et al.*, 2007).

In tumor cells, the stresses can cause disturbances in the protein mechanisms and lead to misfolded and/or unfolded proteins that get accumulated at the ER lumen provoking the ER stress. This situation is mediated by the unfolded protein response (UPR), which is an adaptative response orchestrated by sensor proteins that have the aim

to restore homeostasis, but in a negative case, then cells will be led to apoptosis (Hetz, Chevet and Harding, 2013; Yadav *et al.*, 2014).

The UPR sensor proteins are protein kinase RNA-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1α (IRE1α). In brief, IRE1α when activated will induce the expression of the transcription factor XBP1s, which will target genes to promote protein quality control, folding, degradation, and biogenesis of organelles. In the same way, ATF6 can be processed after translocating to the Golgi apparatus and trigger genes related to UPR control. As for PERK, its activation by autophosphorylation can regulate protein synthesis by phosphorylating the eukaryotic translation initiator factor 2α (eIF2α), which at this state compromise the protein translation, by not binding to translation initial complex and therefore will not recruit the methionyl-initiator tRNA to the 40S ribosomal subunit (Hetz, Chevet and Harding, 2013; Leprivier *et al.*, 2015; Cubillos-Ruiz, Bettigole and Glimcher, 2017).

Another major regulator of protein synthesis is the mammalian target of rapamycin (mTOR) complex. mTOR is a dynamic and multi-protein pathway that can be induced by many other pathways and proteins. It is well known that mTOR plays a role in cell growth, proliferation, metabolism, and survival. And can be stimulated by amino acids, insulin, growth factors and nutrients. This pathway aggregates two main complexes: the mammalian target of rapamycin complex 1 (mTORC1) and complex 2 (mTORC2) (Laplante and Sabatini, 2009; Zou *et al.*, 2020).

The mTORC1 complex regulates protein synthesis for the cap-dependent translation manner. In summary, for a regular synthesis process, a translation repressor protein called Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), a “checkpoint” at initial translation, binds to eIF4E blocking it, which does not allow the recruitment of the 40S machinery to promote translation. If cells need to produce proteins, mTORC1 then promotes disconnection of 4E-BP1 by phosphorylating it, and permit eIF4E to start translation. At the same time, mTORC1 also promotes phosphorylation of the 70 ribosomal protein S6 kinase (p70S6K), which will then induce phosphorylation of its substrate at the 40S unit of the ribosome, the S6 (ribosomal protein, rpS6), and will help modulate the protein translation at the ribosome (Ruvinsky and

Meyuhas, 2006; Laplante and Sabatini, 2009; Silvera, Formenti and Schneider, 2010; Leprivier *et al.*, 2015; Qin, Jiang and Zhang, 2016).

In stress situations like hypoxia, nutrient deprivation and oxidative stress, mTORC1 is inactivated, therefore reducing or stopping mRNA translation, and consequently, reducing global protein synthesis (Silvera, Formenti and Schneider, 2010; Leprivier *et al.*, 2015). However, even though hypoxia inhibits mTOR, and consequently, considerably reducing the protein synthesis, HIF1-α translation continues, and evidence suggests it may occur through a selective translation, in which HIF1-α enables mTOR to selectively keep HIF1-α production, as well as other interesting proteins for tumor development. In addition, inhibition of mTOR, for example, through rapamycin, shows that HIF1-α is also reduced (Wouters and Koritzinsky, 2008; Knaup *et al.*, 2009).

Moreover, over the last decades, evidence, specially in hypoxia situations, suggests that these stress regulators mechanisms, ER stress by UPR and mTOR responses, may act together, and constantly crosstalk. Nevertheless, these pathways can influence cell mechanisms, such as ER homeostasis, metabolism, angiogenesis, and autophagy. For instance, HIF1-α, mTOR and UPR can independently induce autophagy, which may incur that there is a connection between them when hypoxia is ruling the tumor cell (Wouters and Koritzinsky, 2008; Senft and Ronai, 2016).

7. C0NCLUSION

Taken together, the results from this study permits the following conclusions:

1- HT1080 cells acquired a hypoxic phenotype after exposure to chemical hypoxia, induced by cobalt chloride ($CoCl_2$, 100µM), and by low oxygen tension (1%O_2), induced by a hypoxia chamber.
2- HIF1-α is increased by peptide C16 in combination to $CoCl_2$ and 0.5%FBS media, but decreased in 1%O_2 and 10%FBS media.
3- Protein synthesis as well as protein levels are reduced on HT1080 cells in hypoxia condition, and even more diminished by peptide C16, which promotes the same effect in normoxia condition.
4- Reduced protein synthesis may be explained by the disappearance of ribosomal protein S6, a mTORC1 target that controls translation.
5- Cell size and number are decreased when peptide C16 is involved, and may be a direct impact from S6 reduction.
6- Peptide C16 enhances degradation of a fluorescent gelatin in chemical hypoxia, and confirms its performance in normoxia.
7- MMP-2 is activated by the peptide and phosphorylation of GRASP55 is reduced, which can also suggest another sign that ECM is being remodeled in response to peptide C16.

REFERENCES

Ajdukovic, J. (2016) 'HIF-1 - A big chapter in the cancer tale', *Experimental Oncology*, 38(1), pp. 9–12. doi: 10.31768/2312-8852.2016.38(1):9-12.

Alberts, B. *et al.* (2007) *Molecular Biology of the Cell*, *Molecular Biology of the Cell*. doi: 10.1201/9780203833445.

Anderson, N. M. and Simon, M. C. (2020) 'The tumor microenvironment', *Current Biology*, 30(16), pp. R921–R925. doi: 10.1016/j.cub.2020.06.081.

Artym, V. V. *et al.* (2006) 'Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: Defining the stages of invadopodia formation and function', *Cancer Research*, 66(6), pp. 3034–3043. doi: 10.1158/0008-5472.CAN-05-2177.

Artym, V. V. *et al.* (2011) 'Dynamic membrane remodeling at invadopodia differentiates invadopodia from podosomes', *European Journal of Cell Biology*, pp. 172–180. doi: 10.1016/j.ejcb.2010.06.006.

Artym, V. V., Yamada, K. M. and Mueller, S. C. (2009) 'ECM Degradation Assays for Analyzing Local Cell Invasion', in *Methods in molecular biology (Clifton, N.J.)*, pp. 211–219. doi: 10.1007/978-1-59745-413-1_15.

Ashton, N. (1974) 'Vascular basement membrane changes in diabetic retinopathy: Montgomery lecture, 1973', *British Journal of Ophthalmology*, 58(4), pp. 344–366. doi: 10.1136/bjo.58.4.344.

Augoff, K., Hryniewicz-Jankowska, A. and Tabola, R. (2020) 'Invadopodia: clearing the way for cancer cell invasion', *Annals of Translational Medicine*, 8(14), pp. 902–902. doi: 10.21037/atm.2020.02.157.

Augsburger, D. *et al.* (2017) 'Current diagnostics and treatment of fibrosarcoma - perspectives for future therapeutic targets and strategies', *Oncotarget*, 8(61), pp. 104638–104653. doi: 10.18632/oncotarget.20136.

Aumailley, M. *et al.* (2005) 'A simplified laminin nomenclature', *Matrix Biology*, 24(5), pp.

326–332. doi: 10.1016/j.matbio.2005.05.006.

Aumailley, M. (2013) ‘The laminin family’, *Cell Adhesion & Migration*, 7(1), pp. 48–55. doi: 10.4161/cam.22826.

Bahrami, A. and Folpe, A. L. (2010) ‘Adult-type fibrosarcoma: A reevaluation of 163 putative cases diagnosed at a single institution over a 48-year period’, *American Journal of Surgical Pathology*, 34(10), pp. 1504–1513. doi: 10.1097/PAS.0b013e3181ef70b6.

Balbinot-Alfaro, E. *et al.* (2021) ‘Properties, bioactive potential and extraction processes of glycosaminoglycans: An overview’, *Ciencia Rural*, 51(7), pp. 1–9. doi: 10.1590/0103-8478cr20200821.

Balkwill, F. R., Capasso, M. and Hagemann, T. (2012) ‘The tumor microenvironment at a glance’, *Journal of Cell Science*, 125(23), pp. 5591–5596. doi: 10.1242/jcs.116392.

Barillari, G. (2020) ‘The impact of matrix metalloproteinase-9 on the sequential steps of the metastatic process’, *International Journal of Molecular Sciences*. doi: 10.3390/ijms21124526.

Barry-Hamilton, V. *et al.* (2010) ‘Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment’, *Nature Medicine*, 16(9), pp. 1009–1017. doi: 10.1038/nm.2208.

Bergman, A., Condeelis, J. S. and Gligorijevic, B. (2014) ‘Invadopodia in context’, *Cell Adhesion and Migration*, 8(3), pp. 273–279. doi: 10.4161/cam.28349.

Birk, D. E. and Brückner, P. (2011) ‘Collagens, Suprastructures, and Collagen Fibril Assembly’, in *The Extracellular Matrix: an Overview*. doi: 10.1007/978-3-642-16555-9_3.

Blume, P. A. *et al.* (1997) ‘Fibrosarcoma of the foot: A case presentation and review of the literature’, *Journal of Foot and Ankle Surgery*, 36(1), pp. 51–54. doi: 10.1016/S1067-2516(97)80011-7.

Bolós, V. *et al.* (2010) ‘The dual kinase complex FAK-src as a promising therapeutic target in cancer’, *OncoTargets and Therapy*. doi: 10.2147/ott.s6909.

Bonnans, C., Chou, J. and Werb, Z. (2014) ‘Remodelling the extracellular matrix in

development and disease', *Nature Reviews Molecular Cell Biology*, pp. 786–801. doi: 10.1038/nrm3904.

Borriello, L. *et al.* (2020) 'The role of the tumor microenvironment in tumor cell intravasation and dissemination', *European Journal of Cell Biology*. doi: 10.1016/j.ejcb.2020.151098.

Borycki, A. G. (2013) 'The myotomal basement membrane: Insight into laminin-111 function and its control by Sonic hedgehog signaling', *Cell Adhesion and Migration*, 7(1), pp. 72–81. doi: 10.4161/cam.23411.

Bosman, F. T. and Stamenkovic, I. (2003) 'Functional structure and composition of the extracellular matrix', *Journal of Pathology*. doi: 10.1002/path.1437.

Bowman, W. (1840) 'XXI. On the minute structure and movements voluntary muscle', *Philosophical Transactions of the Royal Society of London*, 130, pp. 457–501. doi: 10.1098/rstl.1840.0022.

Braun, M. *et al.* (2019) 'Benign-looking primary fibrosarcoma of the uterus', *Polish Journal of Pathology*, 70(2), pp. 148–152. doi: 10.5114/pjp.2019.87108.

Bray, F. *et al.* (2021) 'The ever-increasing importance of cancer as a leading cause of premature death worldwide', *Cancer*, 127(16), pp. 3029–3030. doi: 10.1002/cncr.33587.

Brihimi-Horn, M. C. and Pouysségur, J. (2009) 'HIF at a glance', *Journal of Cell Science*, 122(8), pp. 1055–1057. doi: 10.1242/jcs.035022.

Bussard, K. M. *et al.* (2016) 'Tumor-associated stromal cells as key contributors to the tumor microenvironment', *Breast Cancer Research*. doi: 10.1186/s13058-016-0740-2.

Byrne, M. B. *et al.* (2014) 'Methods to study the tumor microenvironment under controlled oxygen conditions', *Trends in Biotechnology*, 32(11), pp. 556–563. doi: 10.1016/j.tibtech.2014.09.006.

Cabral-Pacheco, G. A. *et al.* (2020) 'The roles of matrix metalloproteinases and their inhibitors in human diseases', *International Journal of Molecular Sciences*, pp. 1–53. doi: 10.3390/ijms21249739.

Caires-dos-Santos, L. *et al.* (2020) ‘Laminin-derived peptide C16 regulates Tks expression and reactive oxygen species generation in human prostate cancer cells’, *Journal of Cellular Physiology*, 235(1), pp. 587–598. doi: 10.1002/jcp.28997.

Carvalho, M. R. *et al.* (2019) ‘Peptide-Modified Dendrimer Nanoparticles for Targeted Therapy of Colorectal Cancer’, *Advanced Therapeutics*, 2(11), pp. 1–11. doi: 10.1002/adtp.201900132.

Chachami, G. *et al.* (2004) ‘Cobalt induces hypoxia-inducible factor-1α expression in airway smooth muscle cells by a reactive oxygen species- and PI3K-dependent mechanism’, *American Journal of Respiratory Cell and Molecular Biology*, 31(5), pp. 544–551. doi: 10.1165/rcmb.2003-0426OC.

Chen, W.-T. (1989) ‘Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells’, *Journal of Experimental Zoology*, 251(2), pp. 167–185. doi: 10.1002/jez.1402510206.

Choo, A. Y. *et al.* (2008) ‘Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation’, *Proceedings of the National Academy of Sciences of the United States of America*, 105(45), pp. 17414–17419. doi: 10.1073/pnas.0809136105.

Clark, E. S. *et al.* (2007) ‘Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia’, *Cancer Research*, 67(9), pp. 4227–4235. doi: 10.1158/0008-5472.CAN-06-3928.

Clemens, M. J. and Bommer, U. A. (1999) ‘Translational control: The cancer connection’, *International Journal of Biochemistry and Cell Biology*. doi: 10.1016/S1357-2725(98)00127-7.

Cmoch, A., Groves, P. and Pikuła, S. (2014) ‘Biogenesis of invadopodia and their cellular functions.’, *Postepy biochemii*, 60(1), pp. 62–68.

Colognato, H. and Yurchenco, P. D. (2000) ‘Form and function: The laminin family of heterotrimers’, *Developmental Dynamics*, 218(2), pp. 213–234. doi: 10.1002/(SICI)1097-0177(200006)218:2<213::AID-DVDY1>3.0.CO;2-R.

Compton, C. (2021) *Correction to: Cancer: The Enemy from Within*, *Cancer: The Enemy from Within*. doi: 10.1007/978-3-030-40651-6_13.

Cubillos-Ruiz, J. R., Bettigole, S. E. and Glimcher, L. H. (2017) 'Tumorigenic and Immunosuppressive Effects of Endoplasmic Reticulum Stress in Cancer', *Cell*, 168(4), pp. 692–706. doi: 10.1016/j.cell.2016.12.004.

Cui, N., Hu, M. and Khalil, R. A. (2017) *Biochemical and Biological Attributes of Matrix Metalloproteinases*. 1st edn, *Progress in Molecular Biology and Translational Science*. 1st edn. Elsevier Inc. doi: 10.1016/bs.pmbts.2017.02.005.

Czarnowski, D. (2021) 'Syndecans in cancer: A review of function, expression, prognostic value, and therapeutic significance', *Cancer Treatment and Research Communications*, 27, p. 100312. doi: 10.1016/J.CTARC.2021.100312.

Daley, W. P., Peters, S. B. and Larsen, M. (2008) 'Extracellular matrix dynamics in development and regenerative medicine', *Journal of Cell Science*, 121(3), pp. 255–264. doi: 10.1242/jcs.006064.

Danielle A. Murphy, S. A. C. (2012) 'The "ins" and "outs" of podosomes and invadopodia', *Nat Rev Mol Cell Biol.*, 12(7), pp. 413–426. doi: 10.1038/nrm3141.The.

David-Pfeuty, T. and Singer, S. J. (1980) 'Altered distributions of the cytoskeletal proteins vinculin and alpha-actinin in cultured fibroblasts transformed by Rous sarcoma virus.', *Proceedings of the National Academy of Sciences*, 77(11), pp. 6687–6691. doi: 10.1073/pnas.77.11.6687.

Davis, C. K. *et al.* (2019) 'Hypoxia mimetic agents for ischemic stroke', *Frontiers in Cell and Developmental Biology*. doi: 10.3389/fcell.2018.00175.

Denton, A. E., Roberts, E. W. and Fearon, D. T. (2018) 'Stromal Cells in the Tumor Microenvironment', in *Advances in Experimental Medicine and Biology*, pp. 99–114. doi: 10.1007/978-3-319-78127-3_6.

Deryugina, E. I. *et al.* (2001) 'MT1-MMP initiates activation of pro-MMP-2 and integrin αvβ3 promotes maturation of MMP-2 in breast carcinoma cells', *Experimental Cell Research*, 263(2), pp. 209–223. doi: 10.1006/excr.2000.5118.

Díaz, B. *et al.* (2013) ‘Notch increases the shedding of HB-EGF by ADAM12 to potentiate invadopodia formation in hypoxia’, *Journal of Cell Biology*, 201(2), pp. 279–292. doi: 10.1083/jcb.201209151.

Diaz, B. and Yuen, A. (2014) ‘The impact of hypoxia in pancreatic cancer invasion and metastasis’, *Hypoxia*, p. 91. doi: 10.2147/hp.s52636.

Djagaeva, I. and Doronkin, S. (2010) ‘Hypoxia response pathway in border cell migration’, *Cell Adhesion and Migration*, 4(3), pp. 391–395. doi: 10.4161/cam.4.3.11790.

Dubey, P. K., Singodia, D. and Vyas, S. P. (2010) ‘Liposomes modified with YIGSR peptide for tumor targeting’, *Journal of Drug Targeting*, 18(5), pp. 373–380. doi: 10.3109/10611860903483388.

Dvorak, H. F. (2015) ‘Tumors: Wounds that do not heal-redux’, *Cancer Immunology Research*, 3(1). doi: 10.1158/2326-6066.CIR-14-0209.

Eddy, R. J. *et al.* (2017) ‘Tumor Cell Invadopodia: Invasive Protrusions that Orchestrate Metastasis’, *Trends in Cell Biology*, 27(8), pp. 595–607. doi: 10.1016/j.tcb.2017.03.003.

Egeblad, M. and Werb, Z. (2002) ‘New functions for the matrix metalloproteinases in cancer progression’, *Nature Reviews Cancer*, 2(3), pp. 161–174. doi: 10.1038/nrc745.

El-Naggar, A. M. and Sorensen, P. H. (2018) ‘Translational control of aberrant stress responses as a hallmark of cancer’, *Journal of Pathology*. doi: 10.1002/path.5030.

Engbring, J. A. and Kleinman, H. K. (2003) ‘The basement membrane matrix in malignancy’, *Journal of Pathology*, 200(4), pp. 465–470. doi: 10.1002/path.1396.

Engel, J. and Chiquet, M. (2011) ‘An Overview of Extracellular Matrix Structure and Function’, in *The Extracellular Matrix: an Overview*. doi: 10.1007/978-3-642-16555-9_1.

Faisal Khan, K. M. and Falcone, D. J. (1997) ‘Role of laminin in matrix induction of macrophage urokinase-type plasminogen activator and 92-kDa metalloproteinase expression’, *Journal of Biological Chemistry*, pp. 8270–8275. doi: 10.1074/jbc.272.13.8270.

Fingar, D. C. *et al.* (2002) ‘Mammalian cell size is controlled by mTOR and its downstream

targets S6K1 and 4EBP1/eIF4E', *Genes and Development*, 16(12), pp. 1472–1487. doi: 10.1101/gad.995802.

Fingleton, B. and Lynch, C. (2010) 'Cancer in context: Importance of the tumor microenvironment', in *Cell-Extracellular Matrix Interactions in Cancer*. doi: 10.1007/978-1-4419-0814-8_3.

Fletcher, C. D. M. and Unni, K. K. (2002) 'World Health Organization Classification of Tumours Pathology and Genetics of Tumours of Soft Tissue and Bone Edited by', *Cancer*, 177(3).

Fletcher, C. D. M., Unni, K. K. and Mertens, F. (2002) 'Pathology and Genetics of Tumours of Soft Tissue and Bone. WHO Classification of Tumours', *IARCPress Lyon*.

Fletcher CDM, Bridge JA, Hogendoorn PCW, M. F. (2013) *WHO clasification of tumours of soft tissue and bone*, *Lyon: IARC Press*.

Folpe, A. L. (2014) 'Fibrosarcoma: A review and update', *Histopathology*. doi: 10.1111/his.12282.

Folpe, A. L. (2020) '"Hey! Whatever happened to hemangiopericytoma and fibrosarcoma?" An update on selected conceptual advances in soft tissue pathology which have occurred over the past 50 years', *Human Pathology*, 95. doi: 10.1016/j.humpath.2019.10.001.

Franchi, A. and Santucci, M. (2013) 'The contribution of electron microscopy to the characterization of soft tissue fibrosarcomas', *Ultrastructural Pathology*, pp. 9–14. doi: 10.3109/01913123.2012.670017.

Frantz, C., Stewart, K. M. and Weaver, V. M. (2010) 'The extracellular matrix at a glance', *Journal of Cell Science*, pp. 4195–4200. doi: 10.1242/jcs.023820.

Freitas, V. . *et al.* (2004) 'Laminin-1 and SIKVAV a laminin-1-derived peptide, regulate the morphology and protease activity of a human salivary gland adenoid cystic carcinoma cell line', *Oral Oncology*, 40(5), pp. 483–489. doi: 10.1016/j.oraloncology.2003.10.002.

Freitas, V. M. *et al.* (2007) 'SIKVAV, a laminin α1-derived peptide, interacts with integrins

and increases protease activity of a human salivary gland adenoid cystic carcinoma cell line through the ERK 1/2 signaling pathway', *American Journal of Pathology*, 171(1), pp. 124–138. doi: 10.2353/ajpath.2007.051264.

Fu, O. Y. *et al.* (2009) 'Cobalt chloride-induced hypoxia modulates the invasive potential and matrix metalloproteinases of primary and metastatic breast cancer cells', *Anticancer Research*, 29(8), pp. 3131–3138.

Galheigo, M. R. U. . *et al.* (no date) *The laminin-derived peptide C16 is internalized by caveolin-1 coated vesicles and partially directed to lysosomes in MDA-MB-231 breast cancer cell line*.

Gama-de-Souza, L. N. *et al.* (2008) 'Adhesion and protease activity in cell lines from human salivary gland tumors are regulated by the laminin-derived peptide AG73, syndecan-1 and β1 integrin', *Matrix Biology*, 27(5), pp. 402–419. doi: 10.1016/j.matbio.2008.02.007.

Geiger, B. and Yamada, K. M. (2011) 'Molecular architecture and function of matrix adhesions', *Cold Spring Harbor Perspectives in Biology*, 3(5), pp. 1–21. doi: 10.1101/cshperspect.a005033.

Gene Set - HIF1A (no date). Available at: https://maayanlab.cloud/Harmonizome/gene_set/HIF1A/TRANSFAC+Predicted+Transcription+Factor+Targets (Accessed: 23 November 2021).

Gibbs, J., Henderson-Jackson, E. and Bui, M. M. (2016) 'Bone and Soft Tissue Pathology: Diagnostic and Prognostic Implications', *Surgical Clinics of North America*, 96(5), pp. 915–962. doi: 10.1016/j.suc.2016.06.003.

Gilkes, D. M., Semenza, G. L. and Wirtz, D. (2014) 'Hypoxia and the extracellular matrix: Drivers of tumour metastasis', *Nature Reviews Cancer*, 14(6), pp. 430–439. doi: 10.1038/nrc3726.

Goda, N. *et al.* (2003) 'Hypoxia-Inducible Factor 1α Is Essential for Cell Cycle Arrest during Hypoxia', *Molecular and Cellular Biology*, 23(1), pp. 359–369. doi: 10.1128/mcb.23.1.359-369.2003.

Gould, C. M. and Courtneidge, S. A. (2014) ‘Regulation of invadopodia by the tumor microenvironment’, *Cell Adhesion and Migration*, 8(3), pp. 226–235. doi: 10.4161/cam.28346.

Graf, J. *et al.* (1987) ‘Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding’, *Cell*, 48(6), pp. 989–996. doi: 10.1016/0092-8674(87)90707-0.

Grant, D. S. *et al.* (1992) ‘Interaction of endothelial cells with a laminin A chain peptide (SIKVAV) in vitro and induction of angiogenic behavior in vivo’, *Journal of Cellular Physiology*, 153(3). doi: 10.1002/jcp.1041530324.

Guan, B. J. *et al.* (2017) ‘A Unique ISR Program Determines Cellular Responses to Chronic Stress’, *Molecular Cell*, 68(5), pp. 885-900.e6. doi: 10.1016/j.molcel.2017.11.007.

Hajdu, S. I. (1998) ‘Fibrosarcoma: a historic commentary.’, *Cancer*, 82(11), pp. 2081–2089. doi: 10.1002/(SICI)1097-0142(19980601)82:11<2081::AID-CNCR1>3.0.CO;2-Z.

Hall, A. *et al.* (1983) ‘Identification of transforming gene in two human sarcoma cell lines as a new member of the ras gene family located on chromosome 1’, *Nature*, 303(5916). doi: 10.1038/303396a0.

Hallmann, R. *et al.* (2020) ‘The role of basement membrane laminins in vascular function’, *International Journal of Biochemistry and Cell Biology*, 127(August), p. 105823. doi: 10.1016/j.biocel.2020.105823.

Hanahan, D. and Coussens, L. M. (2012) ‘Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment’, *Cancer Cell*, 21(3), pp. 309–322. doi: 10.1016/j.ccr.2012.02.022.

Hanahan, D. and Weinberg, R. A. (2011) ‘Hallmarks of cancer: The next generation’, *Cell*, 144(5), pp. 646–674. doi: 10.1016/j.cell.2011.02.013.

Henke, E., Nandigama, R. and Ergün, S. (2020) ‘Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy’, *Frontiers in Molecular Biosciences*. doi: 10.3389/fmolb.2019.00160.

Hennipman, A. and van Ginneken, P. J. (1985) ‘Fibrosarcoma occurring in scar tissue.’, *The Netherlands journal of surgery*, 37(3), p. 93. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4022423.

Henriet, P. and Emonard, H. (2019) ‘Matrix metalloproteinase-2: Not (just) a “hero” of the past’, *Biochimie*, 166, pp. 223–232. doi: 10.1016/j.biochi.2019.07.019.

Hetz, C., Chevet, E. and Harding, H. P. (2013) ‘Targeting the unfolded protein response in disease’, *Nature Reviews Drug Discovery*, 12(9), pp. 703–719. doi: 10.1038/nrd3976.

Hinshaw, D. C. and Shevde, L. A. (2019) ‘The tumor microenvironment innately modulates cancer progression’, *Cancer Research*, pp. 4557–4567. doi: 10.1158/0008-5472.CAN-18-3962.

Hoffmann, C. *et al.* (2018) ‘Hypoxia promotes breast cancer cell invasion through HIF-1α-mediated up-regulation of the invadopodial actin bundling protein CSRP2’, *Scientific Reports*, 8(1), p. 10191. doi: 10.1038/s41598-018-28637-x.

Hohenester, E. (2019) ‘Structural biology of laminins’, *Essays in Biochemistry*. doi: 10.1042/EBC20180075.

Hohenester, E. and Yurchenco, P. D. (2013) ‘Laminins in basement membrane assembly’, *Cell Adhesion and Migration*, 7(1), pp. 56–63. doi: 10.4161/cam.21831.

Horejs, C. M. *et al.* (2014) ‘Biologically-active laminin-111 fragment that modulates the epithelial-to-mesenchymal transition in embryonic stem cells’, *Proceedings of the National Academy of Sciences of the United States of America*, 111(16). doi: 10.1073/pnas.1403139111.

Horejs, C. M. (2016) ‘Basement membrane fragments in the context of the epithelial-to-mesenchymal transition’, *European Journal of Cell Biology*, 95(11), pp. 427–440. doi: 10.1016/j.ejcb.2016.06.002.

Hoshino, D., Branch, K. M. and Weaver, A. M. (2013) ‘Signaling inputs to invadopodia and podosomes’, *Journal of Cell Science*, 126(14), pp. 2979–2989. doi: 10.1242/jcs.079475.

Hubbi, M. E. and Semenza, G. L. (2015) ‘Regulation of cell proliferation by hypoxia-

inducible factors', *American Journal of Physiology - Cell Physiology*, pp. C775–C782. doi: 10.1152/ajpcell.00279.2015.

Humphrey, J. D., Dufresne, E. R. and Schwartz, M. A. (2014) 'Mechanotransduction and extracellular matrix homeostasis', *Nature Reviews Molecular Cell Biology*, pp. 802–812. doi: 10.1038/nrm3896.

Hynes, R. O. (2009) 'The extracellular matrix: Not just pretty fibrils', *Science*. doi: 10.1126/science.1176009.

Iizuka, S. *et al.* (2020) 'Crosstalk between invadopodia and the extracellular matrix', *European Journal of Cell Biology*, 99(7), p. 151122. doi: 10.1016/j.ejcb.2020.151122.

Itoh, Y. (2015) 'Membrane-type matrix metalloproteinases: Their functions and regulations', *Matrix Biology*, 44–46, pp. 207–223. doi: 10.1016/j.matbio.2015.03.004.

Iwamoto, Yukihide *et al.* (1987) 'YIGSR, a Synthetic Laminin Pentapeptide, Inhibits Experimental Metastasis Formation.', 238, pp. 1132–1135. Available at: https://www.jstor.org/stable/1700857.

Jayadev, R. and Sherwood, D. R. (2017) 'Basement membranes', *Current Biology*, 27(6), pp. R207–R211. doi: 10.1016/j.cub.2017.02.006.

Jeannot, P. and Besson, A. (2020) 'Cortactin function in invadopodia', *Small GTPases*, 11(4), pp. 256–270. doi: 10.1080/21541248.2017.1405773.

Junqueira, L. C. and Carneiro, J. (2013) *Histologia Básica: texto e atlas*, *Histologia Básica*.

Junttila, M. R. and De Sauvage, F. J. (2013) 'Influence of tumour micro-environment heterogeneity on therapeutic response', *Nature*, pp. 346–354. doi: 10.1038/nature12626.

Kai, F. B., Drain, A. P. and Weaver, V. M. (2019) 'The Extracellular Matrix Modulates the Metastatic Journey', *Developmental Cell*, 49(3), pp. 332–346. doi: 10.1016/j.devcel.2019.03.026.

Kaidi, A., Williams, A. C. and Paraskeva, C. (2007) 'Interaction between β-catenin and HIF-1 promotes cellular adaptation to hypoxia', *Nature Cell Biology*, 9(2). doi: 10.1038/ncb1534.

Kalluri, R. (2003) ‘Basement membranes: Structure, assembly and role in tumour angiogenesis’, *Nature Reviews Cancer*, 3(6), pp. 422–433. doi: 10.1038/nrc1094.

Kalluri, R. and Zeisberg, M. (2006) ‘Fibroblasts in cancer’, *Nature Reviews Cancer*, 6(5), pp. 392–401. doi: 10.1038/nrc1877.

Kanemoto, T. *et al.* (1990) ‘Identification of an amino acid sequence from the laminin A chain that stimulates metastasis and collagenase IV production’, *Proceedings of the National Academy of Sciences of the United States of America*, 87(6), pp. 2279–2283. doi: 10.1073/pnas.87.6.2279.

Kang, S. A. *et al.* (2013) ‘mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin’, *Science*, 341(6144). doi: 10.1126/science.1236566.

Kaushik, N. *et al.* (2019) ‘Proinvasive extracellular matrix remodeling for tumor progression’, *Archives of Pharmacal Research*, 42(1), pp. 40–47. doi: 10.1007/s12272-018-1097-0.

Kessenbrock, K., Plaks, V. and Werb, Z. (2010) ‘Matrix Metalloproteinases: Regulators of the Tumor Microenvironment’, *Cell*, 141(1), pp. 52–67. doi: 10.1016/j.cell.2010.03.015.

Kikkawa, Y. *et al.* (2013) ‘Laminin-111-derived peptides and cancer’, *Cell Adhesion and Migration*, 7(1), pp. 150–159. doi: 10.4161/cam.22827.

Kim, W. S., Jung, W. K. and Park, S. J. (2015) ‘Antioxidant Peptide Derived from Spirulina maxima Suppresses HIF1 α-Induced Invasive Migration of HT1080 Fibrosarcoma Cells’, *Journal of Chemistry*, 2015. doi: 10.1155/2015/308602.

Kirkwood, J. M. *et al.* (2012) ‘Immunotherapy of cancer in 2012’, *CA Cancer Journal for Clinicians*, 62(5), pp. 309–335. doi: 10.3322/caac.20132.

Kisling, A., Lust, R. M. and Katwa, L. C. (2019) ‘What is the role of peptide fragments of collagen I and IV in health and disease?’, *Life Sciences*, pp. 30–34. doi: 10.1016/j.lfs.2019.04.042.

Klijanienko, J. and Lagacé, R. (2011) *Soft Tissue Tumors: A Multidisciplinary, Decisional Diagnostic Approach*, *Soft Tissue Tumors: A Multidisciplinary, Decisional Diagnostic*

Approach. doi: 10.1002/9780470947821.

Knaup, K. X. *et al.* (2009) ‘Mutual regulation of hypoxia-inducible factor and mammalian target of rapamycin as a function of oxygen availability’, *Molecular Cancer Research*, 7(1), pp. 88–98. doi: 10.1158/1541-7786.MCR-08-0288.

Kolli-Bouhafs, K. *et al.* (2014) ‘FAK competes for Src to promote migration against invasion in melanoma cells’, *Cell Death and Disease*, 5(8), pp. 1–10. doi: 10.1038/cddis.2014.329.

Kuratomi, Y. *et al.* (2002) ‘Laminin γ1 chain peptide, C-16 (KAFDITYVRLKF), promotes migration, MMP-9 secretion, and pulmonary metastasis of B16-F10 mouse melanoma cells’, *British Journal of Cancer*, 86(7), pp. 1169–1173. doi: 10.1038/sj.bjc.6600187.

Labani-Motlagh, A., Ashja-Mahdavi, M. and Loskog, A. (2020) ‘The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses’, *Frontiers in Immunology*. doi: 10.3389/fimmu.2020.00940.

Laplante, M. and Sabatini, D. M. (2009) ‘mTOR signaling at a glance’, *Journal of Cell Science*, 122(20), pp. 3589–3594. doi: 10.1242/jcs.051011.

Laronha, H. and Caldeira, J. (2020) ‘Structure and Function of Human Matrix Metalloproteinases’, *Cells*. doi: 10.3390/cells9051076.

LeBleu, V. S., MacDonald, B. and Kalluri, R. (2007) ‘Structure and function of basement membranes’, *Experimental Biology and Medicine*, 232(9), pp. 1121–1129. doi: 10.3181/0703-MR-72.

Lee, L. J. *et al.* (2021) ‘Cancer Plasticity: The Role of mRNA Translation’, *Trends in Cancer*, 7(2), pp. 134–145. doi: 10.1016/j.trecan.2020.09.005.

Lee, M. H. and Murphy, G. (2004) ‘Matrix metalloproteinases at a glance’, *Journal of Cell Science*, 117(18), pp. 4015–4016. doi: 10.1242/jcs.01223.

Leong, H. S. *et al.* (2014) ‘Invadopodia Are Required for Cancer Cell Extravasation and Are a Therapeutic Target for Metastasis’, *Cell Reports*, 8(5), pp. 1558–1570. doi: 10.1016/j.celrep.2014.07.050.

Leprivier, G. *et al.* (2015) ‘Stress-mediated translational control in cancer cells’, *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 1849(7), pp. 845–860. doi: 10.1016/j.bbagrm.2014.11.002.

Limon, J. *et al.* (1998) ‘in Two Adult Fibrosarcomas’, 123(June 1997), pp. 119–123.

Linder, S., Wiesner, C. and Himmel, M. (2011) ‘Degrading devices: Invadosomes in proteolytic cell invasion’, *Annual Review of Cell and Developmental Biology*, 27. doi: 10.1146/annurev-cellbio-092910-154216.

Löffek, S., Schilling, O. and Franzke, C. W. (2011) ‘Series “matrix metalloproteinases in lung health and disease” edited by J. Müller-Quernheim and O. Eickelberg number 1 in this series: Biological role of matrix metalloproteinases: A critical balance’, *European Respiratory Journal*, 38(1), pp. 191–208. doi: 10.1183/09031936.00146510.

Lu, P. *et al.* (2011) ‘Extracellular Matrix degradation and remodeling in development and disease’, *Cold Spring Harbor Perspectives in Biology*, 3(12). doi: 10.1101/cshperspect.a005058.

Lucien, F. *et al.* (2011) ‘Hypoxia-induced invadopodia formation involves activation of NHE-1 by the p90 ribosomal s6 kinase (p90RSK)’, *PLoS ONE*, 6(12). doi: 10.1371/journal.pone.0028851.

Lugassy, C. *et al.* (2007) ‘C16 laminin peptide increases angiotropic extravascular migration of human melanoma cells in a shell-less chick chorioallantoic membrane assay’, *British Journal of Dermatology*, pp. 780–782. doi: 10.1111/j.1365-2133.2007.08120.x.

Machesky, L. M. (2008) ‘Lamellipodia and filopodia in metastasis and invasion’, *FEBS Letters*, pp. 2102–2111. doi: 10.1016/j.febslet.2008.03.039.

Magnuson, B., Ekim, B. and Fingar, D. C. (2012) ‘Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks’, *Biochemical Journal*. doi: 10.1042/BJ20110892.

Mak, K. M. and Mei, R. (2017) ‘Basement Membrane Type IV Collagen and Laminin: An Overview of Their Biology and Value as Fibrosis Biomarkers of Liver Disease’, *Anatomical Record*, 300(8), pp. 1371–1390. doi: 10.1002/ar.23567.

Manou, D. *et al.* (2019) ‘The complex interplay between extracellular matrix and cells in tissues’, in *Methods in Molecular Biology*. doi: 10.1007/978-1-4939-9133-4_1.

Martin, K. H. *et al.* (2012) ‘Quantitative measurement of invadopodia-mediated extracellular matrix proteolysis in single and multicellular contexts’, *Journal of Visualized Experiments*, (66), pp. 1–10. doi: 10.3791/4119.

Masi, I. *et al.* (2020) ‘Tumor Cellular and Microenvironmental Cues Controlling Invadopodia Formation’, *Frontiers in Cell and Developmental Biology*. doi: 10.3389/fcell.2020.584181.

Masoud, G. N. and Li, W. (2015) ‘HIF-1α pathway: Role, regulation and intervention for cancer therapy’, *Acta Pharmaceutica Sinica B*, 5(5), pp. 378–389. doi: 10.1016/j.apsb.2015.05.007.

McKee, T. J. *et al.* (2019) ‘Extracellular matrix composition of connective tissues: a systematic review and meta-analysis’, *Scientific Reports*, 9(1), pp. 1–15. doi: 10.1038/s41598-019-46896-0.

Md Hashim, N. F. *et al.* (2013) ‘Hypoxia-induced invadopodia formation: A role for β-PIX’, *Open Biology*, 3(JUN). doi: 10.1098/rsob.120159.

Meyuhas, O. (2015) ‘Ribosomal Protein S6 Phosphorylation: Four Decades of Research’, *International Review of Cell and Molecular Biology*, 320, pp. 41–73. doi: 10.1016/BS.IRCMB.2015.07.006.

Miner, J. H. *et al.* (1997) ‘The laminin α chains: Expression, developmental transitions, and chromosomal locations of α1-5, identification of heterotrimeric laminins 8- 11, and cloning of a novel α3 isoform’, *Journal of Cell Biology*, 137(3), pp. 685–701. doi: 10.1083/jcb.137.3.685.

Miner, J. H. (2011) ‘Basement Membrane’, in *The Extracellular Matrix: an Overview*.

Miner, J. H. and Yurchenco, P. D. (2004) ‘Laminin functions in tissue morphogenesis’, *Annual Review of Cell and Developmental Biology*, 20(M), pp. 255–284. doi: 10.1146/annurev.cellbio.20.010403.094555.

Miura, M. *et al.* (2019) ‘Estrogen producing ovarian fibrosarcoma: A case report’, *Nagoya Journal of Medical Science*, 81(1), pp. 171–176. doi: 10.18999/nagjms.81.1.171.

Mott, J. D. and Werb, Z. (2004) ‘Regulation of matrix biology by matrix metalloproteinases’, *Current Opinion in Cell Biology*, 16(5), pp. 558–564. doi: 10.1016/j.ceb.2004.07.010.

Mouw, J. K., Ou, G. and Weaver, V. M. (2014) ‘Extracellular matrix assembly: A multiscale deconstruction’, *Nature Reviews Molecular Cell Biology*, pp. 771–785. doi: 10.1038/nrm3902.

Muiznieks, L. D. and Keeley, F. W. (2013) ‘Molecular assembly and mechanical properties of the extracellular matrix: A fibrous protein perspective’, *Biochimica et Biophysica Acta - Molecular Basis of Disease*, pp. 866–875. doi: 10.1016/j.bbadis.2012.11.022.

Muñoz-Sánchez, J. and Chánez-Cárdenas, M. E. (2019) ‘The use of cobalt chloride as a chemical hypoxia model’, *Journal of Applied Toxicology*, 39(4), pp. 556–570. doi: 10.1002/jat.3749.

Murphy, D. A. and Courtneidge, S. A. (2011) ‘The “ins” and “outs” of podosomes and invadopodia: Characteristics, formation and function’, *Nature Reviews Molecular Cell Biology*, 12(7), pp. 413–426. doi: 10.1038/nrm3141.

Muz, B. *et al.* (2015) ‘The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy’, *Hypoxia*, p. 83. doi: 10.2147/hp.s93413.

Nagase, H., Visse, R. and Murphy, G. (2006) ‘Structure and function of matrix metalloproteinases and TIMPs’, *Cardiovascular Research*. doi: 10.1016/j.cardiores.2005.12.002.

Nan, J. N., Kim, O. R. and Lee, M. A. (2019) ‘β-Catenin expression is associated with cell invasiveness in pancreatic cancer’, *Korean Journal of Internal Medicine*, 34(3). doi: 10.3904/kjim.2017.155.

Nascimento, C. F. *et al.* (2010) ‘Role of MMP9 on invadopodia formation in cells from adenoid cystic carcinoma. study by laser scanning confocal microscopy’, *Microscopy Research and Technique*, 73(2), pp. 99–108. doi: 10.1002/jemt.20761.

Nascimento, C. F. *et al.* (2011) ‘Laminin-111 derived peptides AG73 and C16 regulate invadopodia activity of a human adenoid cystic carcinoma cell line’, *Experimental Cell Research*, 317(18), pp. 2562–2572. doi: 10.1016/j.yexcr.2011.08.022.

National Institute for Health and Clinical Excellence (2006) *Improving Outcomes for People with Sarcoma*, *Nice*. Available at: http://www.mccn.nhs.uk/userfiles/documents/SarcomaFullGuidance.pdf.

Nazemi, M. and Rainero, E. (2020) ‘Cross-Talk Between the Tumor Microenvironment, Extracellular Matrix, and Cell Metabolism in Cancer’, *Frontiers in Oncology*, 10(February), pp. 1–7. doi: 10.3389/fonc.2020.00239.

Negishi, Y. and Nomizu, M. (2019) ‘Laminin-derived peptides: Applications in drug delivery systems for targeting’, *Pharmacology and Therapeutics*, pp. 91–97. doi: 10.1016/j.pharmthera.2019.05.017.

Neophytou, C. M. *et al.* (2021) ‘The role of tumor microenvironment in cancer metastasis: Molecular mechanisms and therapeutic opportunities’, *Cancers*, 13(9). doi: 10.3390/cancers13092053.

Nomizu, M. *et al.* (1992) ‘The all-D-configuration segment containing the IKVAV sequence of laminin A chain has similar activities to the all-L-peptide in vitro and in vivo’, *Journal of Biological Chemistry*, pp. 14118–14121. doi: 10.1016/s0021-9258(19)49686-5.

Nomizu, M. *et al.* (1995) ‘Identification of cell binding sites in the laminin α1 chain carboxyl-terminal globular domain by systematic screening of synthetic peptides’, *Journal of Biological Chemistry*, 270(35), pp. 20583–20590. doi: 10.1074/jbc.270.35.20583.

Nomizu, M. *et al.* (1997) ‘Identification of cell binding sequences in mouse laminin chain by systematic peptide screening’, *Journal of Biological Chemistry*, 272(51), pp. 32198–32205. doi: 10.1074/jbc.272.51.32198.

Nomizu, M. *et al.* (1998) ‘Cell binding sequences in mouse laminin α1 chain’, *Journal of Biological Chemistry*, 273(49), pp. 32491–32499. doi: 10.1074/jbc.273.49.32491.

Nüchel, J. *et al.* (2021) ‘An mTORC1-GRASP55 signaling axis controls unconventional secretion to reshape the extracellular proteome upon stress’, *Molecular Cell*, 81(16), pp.

3275-3293.e12. doi: 10.1016/j.molcel.2021.06.017.

Al Okail, M. S. (2010) ‘Cobalt chloride, a chemical inducer of hypoxia-inducible factor-1α in U251 human glioblastoma cell line’, *Journal of Saudi Chemical Society*, 14(2), pp. 197–201. doi: 10.1016/j.jscs.2010.02.005.

Om Alblazi, K. M. and Siar, C. H. (2015) ‘Cellular protrusions - Lamellipodia, filopodia, invadopodia and podosomes - and their roles in progression of orofacial tumours: Current understanding’, *Asian Pacific Journal of Cancer Prevention*, 16(6), pp. 2187–2191. doi: 10.7314/APJCP.2015.16.6.2187.

Ortmann, B., Druker, J. and Rocha, S. (2014) ‘Cell cycle progression in response to oxygen levels’, *Cellular and Molecular Life Sciences*. doi: 10.1007/s00018-014-1645-9.

Parker, J. A. and Mattos, C. (2018) ‘The K-Ras, N-Ras, and H-ras isoforms: Unique conformational preferences and implications for targeting oncogenic mutants’, *Cold Spring Harbor Perspectives in Medicine*, 8(8). doi: 10.1101/cshperspect.a031427.

Paz, H., Pathak, N. and Yang, J. (2014) ‘Invading one step at a time: The role of invadopodia in tumor metastasis’, *Oncogene*, 33(33), pp. 4193–4202. doi: 10.1038/onc.2013.393.

Peláez, R. *et al.* (2017) ‘β3 integrin expression is required for invadopodia-mediated ECM degradation in lung carcinoma cells’, *PLoS ONE*, 12(8), pp. 1–23. doi: 10.1371/journal.pone.0181579.

Petrova, V. *et al.* (2018) ‘The hypoxic tumour microenvironment’, *Oncogenesis*, 7(1). doi: 10.1038/s41389-017-0011-9.

Pezzuto, A. and Carico, E. (2018) ‘Role of HIF-1 in Cancer Progression: Novel Insights. A Review’, *Current Molecular Medicine*, 18(6), pp. 343–351. doi: 10.2174/1566524018666181109121849.

Pickup, M. W., Mouw, J. K. and Weaver, V. M. (2014) ‘The extracellular matrix modulates the hallmarks of cancer’, *EMBO reports*, 15(12). doi: 10.15252/embr.201439246.

Pinheiro, J. J. V. *et al.* (2011) ‘Invadopodia proteins, cortactin and membrane type I matrix

metalloproteinase (MT1-MMP) are expressed in ameloblastoma', *Histopathology*, pp. 1266–1269. doi: 10.1111/j.1365-2559.2011.03995.x.

Platé, M., Guillotin, D. and Chambers, R. C. (2020) 'The promise of mTOR as a therapeutic target pathway in idiopathic pulmonary fibrosis', *European Respiratory Review*, 29(157), pp. 1–7. doi: 10.1183/16000617.0269-2020.

Ponce, M. L. *et al.* (1999) 'Identification of endothelial cell binding sites on the laminin γ1 chain', *Circulation Research*, 84(6), pp. 688–694. doi: 10.1161/01.RES.84.6.688.

Ponce, M. L. and Kleinman, H. K. (2003) 'Identification of redundant angiogenic sites in laminin α1 and γ1 chains', *Experimental Cell Research*, 285(2), pp. 189–195. doi: 10.1016/S0014-4827(03)00056-9.

Ponce, M. L., Nomizu, M. and Kleinman, H. K. (2001) 'An angiogenic laminin site and its antagonist bind through the αvβ3 and α5β1 integrins', *The FASEB Journal*, 15(8), pp. 1389–1397. doi: 10.1096/fj.00-0736com.

Puchalapalli, M. *et al.* (2019) 'The Laminin- α 1 Chain-Derived Peptide, AG73, Binds to Syndecans on MDA-231 Breast Cancer Cells and Alters Filopodium Formation', *Analytical Cellular Pathology*, 2019, pp. 1–10. doi: 10.1155/2019/9192516.

Qin, X., Jiang, B. and Zhang, Y. (2016) '4E-BP1, a multifactor regulated multifunctional protein', *Cell Cycle*, pp. 781–786. doi: 10.1080/15384101.2016.1151581.

Quail, D. F. and Joyce, J. A. (2013) 'Microenvironmental regulation of tumor progression and metastasis', *Nature Medicine*, 19(11), pp. 1423–1437. doi: 10.1038/nm.3394.

Rabanal-Ruiz, Y. and Korolchuk, V. I. (2018) 'mTORC1 and nutrient homeostasis: The central role of the lysosome', *International Journal of Molecular Sciences*, 19(3). doi: 10.3390/ijms19030818.

Rasheed, S. *et al.* (1974) 'Characterization of a newly derived human sarcoma cell line (HT-1080)', *Cancer*, 33(4), pp. 1027–1033. doi: 10.1002/1097-0142(197404)33:4<1027::AID-CNCR2820330419>3.0.CO;2-Z.

Revach, O. Y., Grosheva, I. and Geiger, B. (2020) 'Biomechanical regulation of focal

adhesion and invadopodia formation', *Journal of Cell Science*, 133(20). doi: 10.1242/jcs.244848.

Riley, R. S. *et al.* (2019) 'Delivery technologies for cancer immunotherapy', *Nature Reviews Drug Discovery*, pp. 175–196. doi: 10.1038/s41573-018-0006-z.

Ruvinsky, I. and Meyuhas, O. (2006) 'Ribosomal protein S6 phosphorylation: from protein synthesis to cell size', *Trends in Biochemical Sciences*, 31(6), pp. 342–348. doi: 10.1016/j.tibs.2006.04.003.

Sakamoto, N. *et al.* (1991) 'Inhibition of Angiogenesis and Tumor Growth by a Synthetic Laminin Peptide, CDPGYIGSR-NH2', *Cancer Research*, 51(3), pp. 903–906.

Saxena and Jolly (2019) 'Acute vs. Chronic vs. Cyclic Hypoxia: Their Differential Dynamics, Molecular Mechanisms, and Effects on Tumor Progression', *Biomolecules*, 9(8). doi: 10.3390/biom9080339.

Saykali, B. A. and El-Sibai, M. (2014) 'Invadopodia, regulation, and assembly in cancer cell invasion', *Cell Communication and Adhesion*, 21(4), pp. 207–212. doi: 10.3109/15419061.2014.923845.

Schenk, S. and Quaranta, V. (2003) 'Tales from the crypt[ic] sites of the extracellular matrix', *Trends in Cell Biology*, pp. 366–375. doi: 10.1016/S0962-8924(03)00129-6.

Schito, L. and Semenza, G. L. (2016) 'Hypoxia-Inducible Factors: Master Short title: HIFs drive cancer progression Word count of main text: 3471 19', pp. 1–37. Available at: http://www.elsevier.com/open-access/userlicense/1.0/.

Schmidt, H. *et al.* (2002) 'Gains of 12q are the most frequent genomic imbalances in adult fibrosarcoma and are correlated with a poor outcome', *Genes Chromosomes and Cancer*, 34(1), pp. 69–77. doi: 10.1002/gcc.10036.

Seano, G. and Primo, L. (2015) 'Podosomes and invadopodia: Tools to breach vascular basement membrane', *Cell Cycle*, pp. 1370–1374. doi: 10.1080/15384101.2015.1026523.

Semenza, G. L. *et al.* (1994) 'Transcriptional regulation of genes encoding glycolytic

enzymes by hypoxia-inducible factor 1', *Journal of Biological Chemistry*, 269(38), pp. 23757–23763. doi: 10.1016/s0021-9258(17)31580-6.

Semenza, G. L. (2003) 'Targeting HIF-1 for cancer therapy', *Nature Reviews Cancer*, 3(10), pp. 721–732. doi: 10.1038/nrc1187.

Semenza, Gregg L. (2010a) 'Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics', *Oncogene*, 29(5), pp. 625–634. doi: 10.1038/onc.2009.441.

Semenza, Gregg L. (2010b) 'HIF-1: upstream and downstream of cancer metabolism', *Current Opinion in Genetics and Development*, 20(1), pp. 51–56. doi: 10.1016/j.gde.2009.10.009.

Semenza, Gregg L (2010) 'HIF-1: upstream and downstream of cancer metabolism', *Current Opinion in Genetics & Development*, 20(1), pp. 51–56. doi: 10.1016/j.gde.2009.10.009.

Semenza, G. L. (2013) 'HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations', *Journal of Clinical Investigation*, 123(9), pp. 3664–3671. doi: 10.1172/JCI67230.

Semenza, G. L. (2016) 'The hypoxic tumor microenvironment: A driving force for breast cancer progression', *Biochimica et Biophysica Acta - Molecular Cell Research*, 1863(3), pp. 382–391. doi: 10.1016/j.bbamcr.2015.05.036.

Senft, D. and Ronai, Z. A. (2016) 'Adaptive Stress Responses During Tumor Metastasis and Dormancy', *Trends in Cancer*, pp. 429–442. doi: 10.1016/j.trecan.2016.06.004.

Sever, R. and Brugge, J. S. (2015) 'Signal transduction in cancer', *Cold Spring Harbor Perspectives in Medicine*, 5(4). doi: 10.1101/cshperspect.a006098.

Silvera, D., Formenti, S. C. and Schneider, R. J. (2010) 'Translational control in cancer', *Nature Reviews Cancer*, 10(4), pp. 254–266. doi: 10.1038/nrc2824.

Siqueira, A. S. *et al.* (2010) 'Laminin-derived peptide AG73 regulates migration, invasion, and protease activity of human oral squamous cell carcinoma cells through syndecan-1 and β1 integrin', *Tumor Biology*, 31(1), pp. 46–58. doi: 10.1007/s13277-009-0008-x.

Siqueira, A. S. *et al.* (2016) ‘Laminin-111 peptide C16 regulates invadopodia activity of malignant cells through β1 integrin, Src and ERK 1/2’, *Oncotarget*, 7(30), pp. 47904–47917. doi: 10.18632/oncotarget.10062.

de Siqueira, A. S., Freitas, V. M. and Jaeger, R. G. (2022) ‘Proteolytic processing of laminin and the role of cryptides in tumoral biology’, in *Proteolytic Signaling in Health and Disease*. Elsevier, pp. 113–130. doi: 10.1016/B978-0-323-85696-6.00009-3.

Smuczek, B. *et al.* (2017) ‘The laminin-derived peptide C16 regulates GPNMB expression and function in breast cancer’, *Experimental Cell Research*, 358(2), pp. 323–334. doi: 10.1016/j.yexcr.2017.07.005.

Smuczek, B. (2019) *Estudo da clivagem da laminina em tumores de mama, gerando potenciais peptideos bioativos*. Universidade de S’ao Paulo.

Sriram, A., Bohlen, J. and Teleman, A. A. (2018) ‘Translation acrobatics: how cancer cells exploit alternate modes of translational initiation’, *EMBO reports*, 19(10). doi: 10.15252/embr.201845947.

Stawikowski, M. J. and Fields, G. B. (2015) ‘Matrix Metalloproteinases: From Structure to Function’, *Matrix Metalloproteinase Biology*, pp. 1–22. doi: 10.1002/9781118772287.ch1.

Subarsky, P. and Hill, R. P. (2008) ‘Graded hypoxia modulates the invasive potential of HT1080 fibrosarcoma and MDA MB231 carcinoma cells’, *Clinical and Experimental Metastasis*, 25(3), pp. 253–264. doi: 10.1007/s10585-007-9139-x.

Sung, H. *et al.* (2021) ‘Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries’, *CA: A Cancer Journal for Clinicians*, 71(3), pp. 209–249. doi: 10.3322/caac.21660.

Tashiro, K. *et al.* (1989) ‘A synthetic peptide containing the IKVAV sequence from the A chain of laminin mediates cell attachment, migration, and neurite outgrowth’, *Journal of Biological Chemistry*, 264(27), pp. 16174–16182. doi: 10.1016/s0021-9258(18)71604-9.

Teti, G. *et al.* (2018) ‘The hypoxia-mimetic agent cobalt chloride differently affects human mesenchymal stem cells in their chondrogenic potential’, *Stem Cells International*, 2018. doi: 10.1155/2018/3237253.

Theocharis, A. D. *et al.* (2016) ‘Extracellular matrix structure’, *Advanced Drug Delivery Reviews*, 97, pp. 4–27. doi: 10.1016/j.addr.2015.11.001.

Toro, J. R. *et al.* (2006) ‘Incidence patterns of soft tissue sarcomas, regardless of primary site, in the Surveillance, Epidemiology and End Results program, 1978-2001: An analysis of 26,758 cases’, *International Journal of Cancer*, 119(12), pp. 2922–2930. doi: 10.1002/ijc.22239.

Ukawala, M. *et al.* (2012) ‘Laminin receptor-targeted etoposide loaded polymeric micelles: A novel approach for the effective treatment of tumor metastasis’, *Journal of Drug Targeting*, 20(1), pp. 55–66. doi: 10.3109/1061186X.2011.610799.

Unwith, S. *et al.* (2015) ‘The potential role of HIF on tumour progression and dissemination’, *International Journal of Cancer*, 136(11), pp. 2491–2503. doi: 10.1002/ijc.28889.

Visse, R. and Nagase, H. (2003) ‘Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry’, *Circulation Research*, 92(8), pp. 827–839. doi: 10.1161/01.RES.0000070112.80711.3D.

Wang, H. *et al.* (2018) ‘CT and MRI Findings of Soft Tissue Adult Fibrosarcoma in Extremities’, *BioMed Research International*, 2018(July 2004). doi: 10.1155/2018/6075705.

Wang, M. and Kaufman, R. J. (2014) ‘The impact of the endoplasmic reticulum protein-folding environment on cancer development’, *Nature Reviews Cancer*, 14(9), pp. 581–597. doi: 10.1038/nrc3800.

Wang, X. and Proud, C. G. (2006) ‘The mTOR pathway in the control of protein synthesis’, *Physiology*, 21(5), pp. 362–369. doi: 10.1152/physiol.00024.2006.

Weaver, A. M. (2006) ‘Invadopodia: Specialized cell structures for cancer invasion’, *Clinical and Experimental Metastasis*, pp. 97–105. doi: 10.1007/s10585-006-9014-1.

Weaver, A. M. (2008) ‘Invadopodia’, *Current Biology*, 18(9), pp. 362–364. doi: 10.1016/j.cub.2008.02.028.

Wells, J. M., Gaggar, A. and Blalock, J. E. (2015) ‘MMP generated matrikines’, *Matrix Biology*. doi: 10.1016/j.matbio.2015.01.016.

Wengrod, J. C. and Gardner, L. B. (2015) ‘Cellular adaptation to nutrient deprivation: crosstalk between the mTORC1 and eIF2α signaling pathways and implications for autophagy’, *Cell Cycle*, 14(16), pp. 2571–2577. doi: 10.1080/15384101.2015.1056947.

WHO (2021) *World Health Organization*. Available at: https://www.who.int/news-room/fact-sheets/detail/cancer (Accessed: 11 October 2021).

Wight, T. N., Toole, B. P. and Hascall, V. C. (2011) ‘Hyaluronan and the Aggregating Proteoglycans’, in *The Extracellular Matrix: an Overview*. doi: 10.1007/978-3-642-16555-9_5.

Wilson, W. R. and Hay, M. P. (2011) ‘Targeting hypoxia in cancer therapy’, *Nature Reviews Cancer*, 11(6), pp. 393–410. doi: 10.1038/nrc3064.

Wouters, B. G. and Koritzinsky, M. (2008) ‘Hypoxia signalling through mTOR and the unfolded protein response in cancer’, *Nature Reviews Cancer*, 8(11), pp. 851–864. doi: 10.1038/nrc2501.

Xu, J. and Mosher, D. (2011) ‘Fibronectin and Other Adhesive Glycoproteins’, in *The Extracellular Matrix: an Overview*. doi: 10.1007/978-3-642-16555-9_2.

Yadav, R. K. *et al.* (2014) ‘Endoplasmic Reticulum Stress and Cancer’, *Journal of Cancer Prevention*, 19(2), pp. 75–88. doi: 10.15430/JCP.2014.19.2.75.

Yang, L. and Lin, P. C. (2017) ‘Mechanisms that drive inflammatory tumor microenvironment, tumor heterogeneity, and metastatic progression’, *Seminars in Cancer Biology*, pp. 185–195. doi: 10.1016/j.semcancer.2017.08.001.

Yao, Y. (2017) ‘Laminin: loss-of-function studies’, *Cellular and Molecular Life Sciences*. doi: 10.1007/s00018-016-2381-0.

Yoshida, N. *et al.* (1999) ‘The laminin-derived peptide YIGSR (Tyr-Ile-Gly-Ser-Arg) inhibits human pre-B leukaemic cell growth and dissemination to organs in SCID mice’, *British Journal of Cancer*, 80(12), pp. 1898–1904. doi: 10.1038/sj.bjc.6690618.

Yuan, Y. *et al.* (2003) ‘Cobalt inhibits the interaction between hypoxia-inducible factor-α and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-α’, *Journal of Biological Chemistry*, 278(18), pp. 15911–15916. doi: 10.1074/jbc.M300463200.

Yurchenco, P. D. (2011) ‘Basement membranes: Cell scaffoldings and signaling platforms’, *Cold Spring Harbor Perspectives in Biology*, pp. 1–27. doi: 10.1101/cshperspect.a004911.

Zhang, X., Wang, L. and Qu, Y. (2020) ‘Targeting the β-catenin signaling for cancer therapy’, *Pharmacological Research*, 160, p. 104794. doi: 10.1016/J.PHRS.2020.104794.

Zindanci, I. *et al.* (2011) ‘Fibrosarcoma arising from a burn scar’, *European Journal of Dermatology*, 21(6), pp. 996–997. doi: 10.1684/ejd.2011.1505.

Zou, Z. *et al.* (2020) ‘MTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges’, *Cell and Bioscience*, 10(1), pp. 1–11. doi: 10.1186/s13578-020-00396-1.