

Chapter 7

Hyaluronan in the Extracellular Matrix of Hematological and Solid Tumors. Its Biological Effects



Antonella Icardi, Silvina L. Lompartia, Daniela L. Papademetrio, Paolo Rosales, Mariángeles Díaz, Matías A. Pibuel, Laura Alaniz, and Elida Alvarez

Abstract Tumors are most frequently induced by environmental factors with oncogenic potential. Beside tumor cells, cancer tissue contains several nonmalignant cells with well-defined function, embedded into the acellular extracellular matrix playing an important role in tumor progression. The extracellular matrix (ECM) is an indispensable element of all tissues which can modulate cell growth, survival, migration, immune response, and drug resistance. Among its main molecular components are glycosaminoglycans (GAGs) and proteoglycans (PGs), which have been shown to play fundamental roles either in physiological or malignant tissue. An important GAG of the ECM in the tumor microenvironment (TME) is hyaluronic acid, whose synthesis and degradation are altered in a variety of tumors. The biological effects of the abnormal accumulation of HA and the subsequent remodeling of the associated ECM differ according to the type of tumor. In this sense, the ECM-derived HA biological effects in different types of tumors are discussed below in this chapter. Additionally, we make it clear that approaches that interfere with HA metabolism may be a potential therapeutic approach for the treatment of cancer.

A. Icardi · P. Rosales · L. Alaniz (✉)

Laboratorio de Microambiente Tumoral, Centro de Investigaciones Básicas y Aplicadas (CIBA), Universidad Nacional del Noroeste de la Provincia de Buenos Aires (UNNOBA), Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA), UNNOBA-UNSA-CONICET, Junin, Buenos Aires, Argentina
e-mail: ldalaniz@comunidad.unnoba.edu.ar

S. L. Lompartia · D. L. Papademetrio · M. Díaz · M. A. Pibuel · E. Alvarez (✉)

Departamento de Microbiología, Inmunología, Biotecnología y Genética, Cátedra de Inmunología, Universidad de Buenos Aires Facultad de Farmacia y Bioquímica, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Estudios de la Inmunidad Humoral (IDEHU), Junín, CABA, Argentina
e-mail: elialv@ffyb.uba.ar

7.1 Introduction

The tumor microenvironment (TME) is essential part of tumors, being either solid, as carcinomas, sarcomas, lymphomas, or liquid as leukemia. Within this, TME are composed by an extracellular matrix (ECM) which altered composition directly influences in malignant transformation and/or progression. The components from ECM as proteins, glycosaminoglycans (GAGs) and proteoglycans (PGs), have been shown to play fundamental roles in different physiological processes and malignancies. Among the GAGs, HA, which is our molecule of discussion during this chapter, is a key factor that organized the integrity in the ECM. HA is a member of GAGs family and is synthesized by all vertebrate organisms. It is present in almost all mature tissues, within the interstitial matrix but also at intracellular level. Besides, HA has multiple interactions with other proteins and PGs that also impact the function of ECM (Wei et al. 2020). Stavros Garantziotis and Rashmin C. Savani identified at least four distinctive characteristics of HA that defined its mechanism of action, its structure (weight and size), its binding patterns and chemical modifications, its metabolism, and the induction of intracellular signal (Garantziotis and Savani 2019).

The HA's chemical structure is well defined, it is a linear polysaccharide composed of 2000–25,000 repeating disaccharide units [β -D-glucuronic acid (1 \rightarrow 3) and *N*-acetyl-D-glucosamine β (1 \rightarrow 4)] (Fig. 7.1). This large glycosaminoglycan (GAG) exhibits a molecular weight ranging from 10^5 to 107 Da (Laurent and Fraser 1992).

The large quantity of HA in the mammalian ECM impacts in hydration, lubrication, and physical properties of tissues (Toole 2004). In addition, it mediates cell proliferation and differentiation, regulates cell adhesion and motility, making it not only a structural component, but also an effective signaling biomolecule. In many types of tumors, with enhanced HA production, HA surface binding generate a physical barrier, which covers tumor cells and may contribute to the failure of treatments.

HA has a high rate of turnover in vertebrates, for example, in human, 5 g of the 15 g of total body HA renew daily (Stern 2008). It is synthesized by one of the three hyaluronan synthases (Has1/Has2/Has3). These are multipass transmembrane

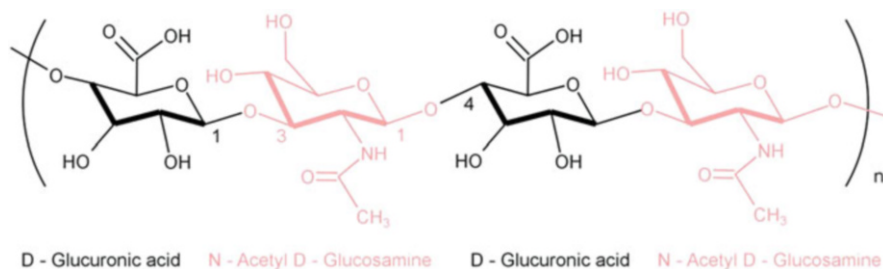


Fig. 7.1 Hyaluronic acid chemical structure. Disaccharide unit [β -D-glucuronic acid (1 \rightarrow 3) and *N*-acetyl-D-glucosamine β (1 \rightarrow 4)] that forms the linear nonsulfated polysaccharide

enzymes that extrude HA while it is being synthesized either onto the cell surface or into the ECM (Monslow et al. 2015). Elevated levels of HA are associated with hyperproliferative and malignant phenotypes in melanomas and various carcinomas (Chanmee et al. 2016).

The breakdown of HA (HMW) in tissues can be accomplished by other mechanisms, either enzymatically by hyaluronidases, or by cleavage that are nonenzymatic oxidation reactions. In cancer cells, the type and levels of hyaluronidases are variable; in some cases, it is elevated, in others suppressed, compared to normal tissues (Toole 2004). A result of hyaluronidase activation, low molecular HA (LMW) is present in the ECM, which promotes inflammation, immune cell recruitment, and the epithelial cell migration. On contrary, elevated HMW-HA production, in the absence of fragmentation, is linked to cancer resistance (Toole 2009). Interestingly, Xiao Tian et al. have been observed that naked mole-rat fibroblasts secrete extremely high-molecular-mass HA, which is over five times larger than human or mouse HA. This sort of high-molecular-mass HA accumulates, abundantly in naked mole-rat tissues, providing cancer resistance and longevity to this species (Tian et al. 2013). Additionally, the authors observed that inhibition of HA synthesis enhances in these cells the susceptibility to malignant transformation.

HA interacts with cell surfaces in at least two ways. First, it can bind to specific cell surface receptors, to induce transduction of several intracellular pathway directly or by interacting with other receptors. HA interacts with many proteins, so termed as hyaladherins, several of which are known as surface receptors. The interaction depends on a binding domain presents every hyaladherins that contains seven nonacidic amino acids. This domain is common to all CD44 isoforms (CD44s and CD44v), the lymphatic vessel endothelial HA receptor-1 (LYVE-1), RHAMM (Receptor for hyaluronate-mediated motility), HARE (Hyaluronan receptor for endocytosis), and TOLL4, Stabilin (Orian-Rousseau 2010). Interestingly, layilin is a transmembrane protein widely expressed in different cell types and tissue extracts. It is found in peripheral ruffles of spreading. Layilin colocalizes with talin in ruffles and binds to talin's ~50-kDa head domain (amino acids 280–435), thus represents a membrane-binding site for talin in cell ruffles (Bono et al. 2001). However, this protein has not sequence homology to the other HA receptors and represents a novel member of the HA-binding protein family (Bono et al. 2001).

Among them, CD44 and RHAMM are the best characterized receptors to date as modulators in cancer. CD44 is a single-pass, glycosylated class-I transmembrane protein involved in multiple cellular functions, including interaction with the matrix microenvironment and intracellular signaling. The extracellular portion of CD44 primarily binds to glycosaminoglycan HA, thereby contributing to cell adhesion, migration, angiogenesis, inflammation, wound healing, and downstream signaling that promotes cell growth and survival (Ponta et al. 2003; Nikitovic et al. 2015; Karamanos et al. 2021).

Many studies have found a correlation between HA expression and the malignant properties of diverse tumors, and disruption of HA production or receptor interaction decreased tumor growth (Hosono et al. 2007; Stern 2008). In fact, the interaction of HA with its receptors drives to the activation of oncogenic pathways as the MAP

kinases and PI3K kinase/Akt inducing survival and cell proliferation, as well as various transport that participate in drug resistance and malignant cell properties (Toole and Slomiany 2008; Bouguignon 2009). Various adaptor proteins such as Vav2, Grb2, and Gab-1 mediate interaction of CD44 with upstream effectors as RhoA, Rac1, and Ras, which drive these pathways (Bouguignon 2008).

7.1.1 HA and Tumor Progression

HA participates in cancer initiation and tumor progression (Chanmee et al. 2016). The increased deposition of HA in tumor tissues is not an inactive process, rather, it triggers signaling events and promotes the association between CD44 and other cell surface receptors, driving to the activation of antiapoptotic pathways mediated by receptor tyrosine kinases that finally promote survival of tumor cells (Bouguignon 2008). Therefore, increase of HA relates to poor patient prognosis and facilitates tumor proliferation, invasion, and angiogenesis among others (Toole and Slomiany 2008). However, contradictory data also exist. In some contexts, accumulation of HA decreases tumorigenicity (Chanmee et al. 2016), while the expression of hyaluronidases, enzymes that degrade HA, can stimulate dissimilar tumor behavior (Stern and Jedrzejewski 2006).

HA Oligosaccharides (oHA) were reported to have suppressive effects on various malignant tumors via disruption of the interactions between HA and receptors. oHA has been shown to suppress PI3K/Akt signaling pathway and module cell behavior as growth, cell survival, and expression of multidrug-resistance genes. In some lymphoma model as well as in breast carcinoma, oHA were able to induce apoptosis and reduce tumor growth in vivo (Russo et al. 2008). Besides, it was reported that oHA disaccharides suppressed progression of bone metastasis in breast cancer (Urakawa et al. 2012).

Contrary, HMW-HA or native HA contributes to flexibility of extracellular matrices (Solis et al. 2012) which is important for regulating cell trafficking. Moreover, HA contributes to tumor resistance in normal tissues by anti-inflammatory and antiproliferative effects (Cowman et al. 2015). HMW-HA present in either the peri-tumoral stroma or tumor parenchyma possess several functions that favor tumor growth by protection from apoptosis or inhibition of autophagy, not only by stopping the synthesis of autophagosomes but also inducing their degradation (Kuang et al. 2007). Moreover, LMW-HA can activate signaling cascades that promote proliferation, cell migration, neo-angiogenesis, immune cell influx, and mesenchymal cell trafficking (Cyphert et al. 2015). LMW-HA also attracts macrophages, which in the microenvironment, polarize into subpopulation M2 that protect tumor cells from adaptive immune cell killing (Kuang et al. 2007). However, the HA MW-dependent actions can be controversial and are associated with the tumor context.

In the next sections, we will discuss into the different studies that demonstrate the participation of HA in tumor progression, making a distinction between solid tumors

and those of hematological origin, considering as liquids leukemias and myelomas, which do not produce solid masses, and lymphoma.

7.2 HA in Hematological Malignancy

Hematologic malignancies comprise a complex and diverse group of pathologies, including leukemia, myelomas, lymphomas that affect blood-forming cells. In this section, the features of each pathology will be summarized first, and then, the impact of HA and its main receptors CD44 and RHAMM on the progression of each kind of hematological cancer will be described.

7.2.1 HA and Leukemia

Leukemia is an uncommon and heterogeneous group of diseases characterized by infiltration of the bone marrow, blood, and visceral organs by neoplastic cells of the hematopoietic system (Menghrajani and Tallman 2018). Leukemia is among the ten types of cancer with high number of death in both sexes and all ages, according to The Globocan Cancer Observatory (<https://gco.iarc.fr/today/data/factsheets/cancers/36-Leukaemia-fact-sheet.pdf>). In general, leukemia is classified into lymphoid or myeloid (depending on the cell line of origin) and acute or chronic (according to the type of evolution), thus forming four main groups: Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Chronic Myeloid Leukemia (CML).

The ECM of the bone marrow (BM) is the largest anatomical component which contains factors that contribute to adhesion, functionality and even regulation of the hematopoietic stem cells (HSC) (Karantanou et al. 2018). In this way, HA, as part of this ECM, actively contributes to these effects. A decrease in the levels of HA in the BM impairs the ability of microenvironment to maintain normal hematopoiesis, and recruit mesenchymal hematopoietic cells into the medulla in alive (Goncharova et al. 2012; Khaldoyanidi et al. 2014). Similarly, the HA would appear to be critical in the niches of these cells as it would protect them from DNA damage by activating efflux pumps capable of extruding genotoxic compounds (Csoka and Stern 2013). In addition, the viscoelastic properties of HA contribute to the porosity and malleability of the ECM of stem cell niches which is important to resist somatic mutations and mechanical damage (Simpson et al. 2016; Gesteira et al. 2017). However, these properties are also used by tumor cells to establish multidrug resistance (García et al. 2009; Lomparđía et al. 2013).

ALL results from the acquisition of mutations in hematopoietic progenitors that confer a proliferative and/or survival advantage and impair hematopoietic differentiation (Ley et al. 2013). Genetics is considered one of the main players in the etiology of acute leukemia (Tebbi 2021). However, other factors, such as age,

environment and occupation, infections, radiation, among others, have been implicated in leukemogenesis (Tebbi 2021). Interestingly, recent studies suggested that leukemia results from a type of gene–environmental interaction that can cooperate with a genetic predisposition, not by inducing mutations, but by reprogramming the epigenome to modulate gene expression (González-Herrero et al. 2018). ALL is diagnosed by the detection of equal or higher than 20% of blasts in the peripheral blood or bone marrow. However, this determination may not accurately define the biology of the disease (Menghrajani and Tallman 2018).

ALL is a hematological malignancy originating from B- or T-lymphoid progenitor cells and represents the most common type of cancer in children and adolescents (80% of cases). The 5-year survival rates of these groups of diseases have increased from 10 to 85% in the last 50 years. However, there are a significant number of relapses and patients who do not respond to therapy. In this sense, 20% of pediatric patients die from the disease and this number rises to 50% in adults (Terwilliger and Abdul-Hay 2017). The B-cell subtype (B-ALL) accounts for about 75–80% of ALL cases and it mainly develops in children with a peak incidence of around 2–5 years (Simioni et al. 2021). T-ALL represents 15–25% of all cases of acute leukemia of children and adults, respectively. Despite being the least frequent subtype, it results to be more aggressive than B-ALL and is considered a risk factor for poor prognosis for patients (Vadillo et al. 2018; Follini et al. 2019).

AML constitutes 15–20% of leukemia in children and adolescents and 80% of acute leukemia in adults (Fiegl 2016). There is a complex classification of AML that created by the combination of clinical, morphologic, immunophenotypic, and genetic features (according with WHO).

Both in bone marrow samples and in the serum of patients diagnosed with acute leukemia, HA levels are increased compared to samples of healthy patients. For this reason, HA has been proposed as a prognostic and follow-up marker for early detection of relapse (Sundström et al. 2005, 2010; Anagnostopoulou et al. 2017). CD44 and RHAMM are the HA receptors most studied in the context of ALL. CD44-HA interactions play a key role in the adhesion, homing, and migration of leukemia initiating cells in bone marrow niches (Zöller 2015; Schepers et al. 2015). Moreover, the ability of CD44 to interact with different components of the ECM is used by leukemic cells to maintain their niches favoring disease relapse and chemoresistance (Hanke et al. 2014; Zöller 2015; Izzi et al. 2017). Indeed, clinical experience indicates that high levels CD44 are associated with a poor prognosis and high relapse rates in AML patients (Hanke et al. 2014). In line with this, it was suggested that CD44 binds to HA promoting an inside-out activation of VLA-4 in AML cells, causing clustering and stabilization of the integrin, leading to the interaction with VCAM-1 in stromal cells. This AML cell–stromal cell interaction triggers survival signaling involving Akt, MAPK, and NF- κ B pathway activation (Gutjahr et al. 2020). NOTCH1 plays a crucial role in T-ALL pathogenesis, as illustrated by the fact that over 60% of human T-ALLs contain gain-of-function NOTCH1 mutations that lead to ligand-independent NOTCH1 signaling (García-Peydró et al. 2018). In human T-ALL xenografts, it was demonstrated that CD44 is a direct NOTCH1 transcriptional target that mediates crucial cell interactions with the

BM microenvironment that result in preleukemic engraftment and further support T-ALL LIC (Leukemic Incited Cell) activity and disease progression (García-Peydró et al. 2018). For this reason, targeted therapy against CD44 aiming to block its interaction with ECM or decrease its expression in tumor cells has been studied (Gul-Uludağ et al. 2014; Vey et al. 2016; Amanzadeh et al. 2017). However, it is well known that different sizes of HA display different biological effects, it has been observed in B-precursor leukemia cells with high surface CD44 expression, that high levels of ultra LMW-HA (<10 kDa) trigger necrosis (Kasai et al. 2017).

Besides, RHAMM is considered a leukemia-associated antigen (Greiner et al. 2002) and it was proposed as an important molecular target for design of therapeutic vaccines in patients with AML (Schmitt et al. 2008; Willemen et al. 2016). A recent study showed that acute leukemia patients with high percentages of RHAMM-positive blasts had more postinduction blasts, blasts in minimal residual disease, and poorer prognosis (Shalini et al. 2018). However, little is known about the implication of this receptor in the biological mechanisms that contribute to the progression of the disease. Similarly, CD38 has become an interesting target for the treatment of ALL and HA its ligand (Lato et al. 2021). Daratumumab, a human monoclonal antibody that binds specifically to CD38, in addition to standard chemotherapy, is under investigation in a phase II trial for pediatric and young adult participants with relapsed and/or refractory T- or B-cell ALL (Clinical Trials. gov identifier: NCT03384654) (Lato et al. 2021). However, the implication of its interaction with HA in the progression of acute leukemia is an empty field for future research.

CLL is the most common leukemia in older patients. TP53 aberrations were associated with aggressiveness and resistance to therapy. For that reason, different first-line therapeutic strategies are used depending on the presence of mutation in TP53, as well as physical fit of patients (Hallek et al. 2018). However, the micro-environment also enhances CCL progression.

Certainly, *ex vivo* assays have established that CLL cells require immune or stromal cells in order to not die by apoptosis, showing the importance of CCL cells and their milieu interaction for leukemic cell survival (Collins et al. 1989). Moreover, HA and its receptors, CD44 and RHAMM, participate in CLL progression. Both CD44 and RHAMM were found to be increased in patients with CLL and have been associated with a worse prognosis, which is why they have been proposed as therapeutic targets (Giannopoulos et al. 2009; Tabarkiewicz and Giannopoulos 2010; Gutjahr et al. 2015). It was described that HA-RHAMM interaction, in presence of IL-8, is able to induce CCL cells migration (Till et al. 1999). Likewise, activation of CD40 enhances CD44 adhesion to HA promoting leukemic cells retention in lymph nodes and enhancing their proliferation and survival (Girbl et al. 2013). Moreover, CD44 is able to complex with key prognostic factors of CLL (CD38 and CD49d) providing a possible nexus between prognosis and leukemic biology (Gutjahr et al. 2015).

On the other hand, CML is a myeloproliferative neoplasm. It is characterized by the presence of Philadelphia chromosome, a product of the reciprocal translocation between chromosomes 9 and 22 (Apperley 2015). This aberration gives rise to a

fusion gene called BCR-ABL which encodes a constitutively activated kinase capable of activating multiple signaling pathways initiating the leukemogenic process due to the clonal expansion of malignant cells. Although tyrosine kinase inhibitor (TKIs) are very effective drugs and significantly increase the mean survival of patients, in many cases, the prolonged use of them leads to the selection of resistant leukemic cells by BCR-ABL-dependent mechanisms (gene amplification, protein overexpression, mutations in the active site of the enzyme) as well as by independent mechanisms (such as Pgp, PI3K, and ERK activation) (Bavaro et al. 2019). However, not only these stimuli favor drug resistance, but also the interaction with the bone marrow milieu enhances CML progression. In this sense, Graham et al. 2002 described that the leukemic stem cells of CML patients were resistant to imatinib and this resistance would be based on the niche in which they are found, which prevents the entry of drug, as well as provides survival signaling (Graham et al. 2002; Schepers et al. 2013).

Regarding HA, it also plays an important role in the progression of CML. In patients with therapeutic failure, after prolonged treatment with imatinib, a gelatinous transformation of the bone marrow characterized by high levels of HA has been described (Hong et al. 2010). In agreement, *in vitro* studies demonstrated that CML cell lines synthesize HA which interacts with CD44 and RHAMM, triggering PI3K/Akt and MEK/ERK signaling pathways avoiding the induction of senescence and favoring resistance to vincristine and imatinib (mediated by PI3K and Pgp activation) (Lomparđía et al. 2013, 2019). It is worth to note that such proteins are involved in BCR-ABL-independent resistance to imatinib. Furthermore, the use of HA oligosaccharides (which are capable of binding to receptors without crosslinking them) allowed the sensitization of CML cell lines to imatinib by inducing apoptosis, as well as senescence through PI3K inhibition (Lomparđía et al. 2016). In the same way, the inhibitor of HA synthesis, 4-methylumbelliferone, synergizes with imatinib inhibiting CML cell lines proliferation and enhances the induction of senescence (Lomparđía et al. 2017, 2019). Considering the gelatinous transformation of bone marrow observed in patients with therapeutic failure and the *in vitro* findings described, it could be suggested that HA participates in resistance to the first-line drug. This appreciation is in concordance with *in vitro* studies which show that HA treatment was able to counteract the antiproliferative and pro-senescent effect of imatinib (Lomparđía et al. 2019). In the same way, CD44 and RHAMM expression have been associated with worse prognostic and TKI resistance in CML patients (Greiner et al. 2002; Hu and Li 2016; Zhou et al. 2017). Therefore, reducing HA levels or mitigating its effects blocking CD44 and RHAMM would be a promising therapeutic approach to improve current therapy.

Considering that the bone marrow niche provides a sanctuary for leukemic stem cells since it favors immune and chemotherapeutic resistance, the looking for improvement in the leukemic therapy could result from understanding this complex microenvironment in which HA is a key player. In this way, the high levels of HA in bone marrow and the background described, let us to hypothesize that the leukemic cells are in a microenvironment that feedback their survival, favoring disease progression.

7.2.2 *HA and Multiple Myeloma*

Multiple myeloma (MM), a plasma cell neoplasia characterized by clonal proliferation of malignant plasma cells mainly in the bone marrow (BM). MM is an aggressive malignancy characterized by the clonal expansion of terminally differentiated B cells in the BM. It is the second most common hematological cancer. MM is clinically defined by increased BM plasmacytosis, serum and/or urine monoclonal immunoglobulin, secretion of free light chains, hypercalcemia, renal insufficiency, anemia, and bone pain due to osteolytic disease (Palumbo and Anderson 2011; Rajkumar et al. 2014).

Plasma cell's (PC) survival depends on different signals from neighboring cells within the BM (Slifka and Ahmed 1998). It was suggested that both the myeloma cells and the microenvironment have undergone alterations as early as during precursor stages of the disease (García-Ortiz et al. 2021). In addition, MM is associated with immune deficiencies, suggesting that the evolution of the disease from a precursor state is related with an immunosuppressive environment that allows tumor growth.

The interaction among myeloma cells and the components of the microenvironment is considered crucial in multiple myeloma pathogenesis. Myeloma cells make use of the supportive surrounding stromal cells, osteocytes, and endothelial cells for their own growth. Myeloma precursor cells have been shown to mediate progressive growth using *in vivo* experimental models that establish the relationship between the microenvironment and tumor signals in regulating tumor growth (Das et al. 2016). Adhesion molecules, cytokines, and the ECM play a critical role in the interplay among genetically transformed clonal plasma cells and stromal cells, leading to the proliferation, progression, and survival of myeloma cells.

The bone marrow microenvironment offers a structure, the ECM, which acts as a feeder and support for myeloma progenitor cells. ECM is constituted by fibronectin, collagen, osteopontin, HA, and laminin. Myeloma cells binding into ECM components proved to be important for survival and drug resistance (Katz 2010).

The glycosaminoglycan HA is a critical component of the hematopoietic microenvironment as mentioned above. It is possible that HA binds either growth factors or other ECM components. HA-expressing stromal cells create hematopoietic foci and act as feeder of hematopoietic cells *in vitro* (Goncharova et al. 2012). Moreover, B cells from MM patients showed HA matrix around the cells.

CD44/HA interactions are implicated in the regulation of homing of normal HSC and malignant cells into BM (Avigdor 2004; Krause et al. 2006). While myeloma cells migrate to the BM (Cook et al. 1997), their interactions with the microenvironment may significantly influence disease progression. CD44 and RHAMM are the major HA receptors of multiple myeloma cells. CD38, another hyaluronic acid interacting protein, is also expressed in high levels in myeloma cells (Costa et al. 2019). On the contrary, its expression is low in other lymphoid and myeloid cells making it an effective target for immunotherapies.

Vincent and Mechti demonstrate that IL-6, a growth factor for myeloma cells, greatly increases CD44 gene expression. The authors shown that IL-6 modulates CD44 RNA alternative splicing and induces the overexpression of all CD44 variant exons. As IL-6 secretion, induced from bone marrow stromal cells by myeloma cells, is mediated through direct cell-to-cell communication involving CD44 adhesion molecules. As consequence, it was suggested that a CD44/IL-6 amplification loop plays a crucial role in myeloma cell survival (Vincent and Mechti 2004).

In addition, adhesion of myeloma cells to the ECM increases angiogenesis, and the expression of adhesion molecules such as VLA4, LFA1, and CD44 are implicated in this event (Vacca et al. 1995). In some cases, the myeloma cells are able to degrade basement membrane and ECM. They must also reduce their affinity to ECM and cells, which are specific to the bone marrow, soon after upregulate migratory proteins. Subsequently, they alter their adhesion properties to extravasate and migrate through the ECM. Besides, in myeloma, plasma cells decrease expression of CD56 while increasing expression of certain CD44 isoforms that are important for proliferation and motility (Dahl et al. 2002). Finally, myeloma cells must degrade the basement membrane to allow passage through gaps of endothelial cells, and when arriving into the circulation, they may penetrate through the vasculature and form tumors in organs. Extramedullary growth is a feature of advanced MM but circulating tumor cells can also be detected even in early stages of MM.

The role for CD44 as a promoter of cell adhesion-mediated drug resistance has been described in several cancers, and it may in part mediate dexamethasone resistance in myeloma (Ohwada et al. 2008).

Lenalidomide therapy is used in frontline, relapsed/refractory, and maintenance settings for multiple myeloma. Lenalidomide-resistant models were found to overexpress the HA. In addition, resistant cells were more adhesive to bone marrow stroma and HA-coated plates. Blockade of CD44 with monoclonal antibodies, free HA, or CD44 knockdown reduce cellular adhesion and sensitized to lenalidomide by affecting Wnt/ β -catenin signal. Bjorklund et al. showed a strong association between CD44 expression and clinical resistance to lenalidomide, suggesting that CD44 levels could serve as biomarkers of lenalidomide resistance (Bjorklund et al. 2014).

Inhibitions of the interactions of MM cells with the BM represent an interesting therapeutic strategy in MM (Bjorklund et al. 2019). The development of new tumor therapies uses HYAL as an adjuvant, agent to depolymerize HA from the ECM. As consequence of the degradation of HA, the absorption of the chemotherapeutic agent of choice is favored. To that end, the administration of Darzalex uses daratumumab plus human recombinant HYALs to treat multiple myeloma (Kaul et al. 2021).

7.2.3 HA and Lymphoma

Lymphoma is a group of hematological malignancies that begins in cells of the lymphoid system. The two main types are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). The majority of lymphoma cases derive from the

B cell lineage and 90% of them originate from different stages of maturation and differentiation during their transit through the germinal center (GC) (Hamel et al. 2012). HL and different subtypes of NHL, such as Diffuse Large B Cell (DLBCL), Burkitt's Lymphoma (BL), and Follicular Lymphoma (FL), derive from this compartment (Klein and Dalla-Favera 2008).

It has been observed that an increase in the HA content in patient biopsies would correlate with high-grade lymphomas (Bertrand et al. 2005), indicating that different types of lymphoma cells produce dissimilar levels of HA and this fact would be related to their malignancy. Additionally, it has been demonstrated that lymphoma cells synthesize this GAG (Funamoto et al. 2002; Kuwabara et al. 2003). Among HA receptors, CD44 has been proposed as a diagnostic marker, as well as a therapeutic target in certain types of lymphomas (Ma et al. 2008; Eberth et al. 2010). In murine NHL lymphoma cell lines, the interaction of HA with CD44 would activate the NF- κ B pathway and this would correlate with an increase in MMP-9 activity, which would favor cell invasion (Alaniz et al. 2004). Moreover, the inhibition of HA synthesis by 4-methylumbelliferone (4-MU) suppresses MMP-9 activity on lymphoma cells (Nakamura et al. 2007). On the other hand, blocking CD44-HA interaction with HA oligosaccharides inhibits the activation of the PI3K/Akt pathway and modulates efflux pumps sensitizing lymphoma resistant cells to chemotherapy treatment (Russo et al. 2008). In contrast, exogenous LMW-HA might increase intracellular doxorubicin accumulation in CD44 positive lymphoma cells by the modulation of ABC drug transporters expression involved in drug efflux in hematopoietic malignancies (Vitale et al. 2018). These data suggest that HA-CD44 interactions also play a key role in invasion and chemoresistance events in lymphoma and that different sizes of HA may exert dissimilar biological effects on lymphoma progression.

In cHL, HRS cells actively modulate their microenvironment to support their survival and proliferation, and to create an immunosuppressive environment (Weniger and Küppers 2021). In this context, it was suggested that HRS cells activates endothelial cells to increase HA levels in the perivascular zone which facilitate the recruitment of naïve T cells into lymph nodes through CD44-HA adhesive pathways (Fhu et al. 2014). Moreover, it was demonstrated that a downregulation of CD44 after inhibition of NF- κ B signaling may decrease cHL cells adhesion to HL-associated fibroblast, highlighting the role of this receptor in with chemotherapy resistance (Celegato et al. 2014).

The literature regarding the role of HA in lymphoma progression is scarce. However, the interplay between HA and CD44 represent an interesting starting point to delve further in the studies of the biology of this GAG in the context of lymphoma and to investigate its potential as a new therapeutic target for this disease.

7.3 Biological Effects of HA from ECM in Solid Tumors: Carcinomas, Sarcomas, and Gliomas

Malignant transformation of epithelial cells results in the development of carcinomas, being the most frequent solid tumors. Sarcomas arise from bone and connective tissues, being the osteosarcoma the most common malignancy of bone cells. Gliomas, the primary brain tumors, are caused by decontrolled growth of glial cells, which are the supporting cells of neurons. In this section, we describe and discuss the role of HA in the most frequent solids tumor mentioned.

7.3.1 Carcinomas

7.3.1.1 HA and Breast Cancer

After lung cancer, breast carcinomas are the second most leading cause of mortality in females and correspond to the most frequently diagnosed malignancy, affecting 2.3 million new cases each year. Hyaluronic acid (HA) is known to be involved in breast cancer progression to metastasis (Toole 2004; Eberth et al. 2010). In breast cancer, a dramatic HA increase is observed, and HA levels have been suggested to predict clinical outcome. Even more, it has been observed that high levels of HA, and in particular small HA oligosaccharides, are associated with poor prognosis (Auvinen et al. 2000) and have been detected that invasive breast cancer cells synthesize and accumulate larger amounts of HA compared to normal breast epithelial tissues (Li et al. 2007). In line with these antecedents, Corte et al. have compared the HA accumulation in early and later stage breast tumors, specifically in ductal carcinoma in situ (DCIS), DCIS with microinvasion and invasive carcinoma, for determining if altered HA production is linked to invasion events in breast cancer. They demonstrated that HA levels are significantly increased in late-stage DCIS compared to early-stage, suggesting a key role for this glycosaminoglycan in the early invasive stage of breast carcinomas (Corte et al. 2010). These antecedents added to the key role of HA in modulating the inflammation, angiogenesis and fibrosis in breast cancer (Heldin et al. 2013), processes involved in its progression to metastasis (Toole 2004). Thus the regulation of HA metabolism will be a potential therapeutic target for the treatment of breast cancer. Numerous studies have demonstrated that inhibition of HA synthesis reduces breast cancer tumor cell proliferation and migration (Kultti et al. 2009; Urakawa et al. 2012; Brett et al. 2018). Brett et al. showed that the invasive and metastatic potential of breast carcinoma cells correlates to the presence of a HA-rich pericellular matrix, and that inhibition of HA synthesis significantly inhibits carcinoma cell extravasation and invasion (Brett et al. 2018). Kultti et al. showed that the growth of MCF-7 cells is sensitive to inhibition of HA synthesis, and that inhibiting HA production inhibits migration of the noninvasive MCF-7 breast cancer cells (Kultti et al. 2009). Even more, Urakawa showed that

suppression of HA synthesis and accumulation by 4-MU inhibited cell proliferation, motility and invasiveness, and induced apoptosis in the highly aggressive MDA-MB-231 breast cancer cell line (Urakawa et al. 2012). Furthermore, inhibition of HA production or its degradation was shown to improve drug delivery in breast cancer (Shpilberg and Jackisch 2013; Kohli et al. 2014; Clift et al. 2019). In this sense, has been shown that the use of hyaluronidases to degrade HA in the TME improves drug delivery of antibody-based trastuzumab-targeted therapy in HER2+ breast cancer (Shpilberg and Jackisch 2013). Similarly, Kohli et al. showed a more heterogeneous distribution of Doxil and reduced tumor growth by inhibiting of HA synthesis (Kohli et al. 2014). Even more, a recent study has shown that HA degradation by pegrhyaluronidase allows to remodel the TME in a murine model of breast cancer increasing the uptake of anti-Programmed Death-Ligand 1 (PD-L1) therapeutic antibody and reducing the tumor growth (Clift et al. 2019). It has also been shown that HA allows to overcome the pro-apoptotic effects of SP-D surfactant protein D and can be used as an escape mechanism in breast cancer. In this sense, when a possible interaction between SP-D, with immune surveillance function against tumor cells, and hyaluronic acid, was studied, it was shown that, in the presence of HA, rfhSP-D is incapable of inducing apoptosis in triple positive breast cancer cell lines that overexpress HER2. In addition, HA-bound rfhSP-D was able to restore cell growth, suggesting that these breast cancer cells may use HA as an escape mechanism to overcome pro-apoptotic effects of surfactant protein D rfhSP-D (Murugaiah et al. 2020). Even more, Witschen et al. found that production of the chemokine CCL2 by breast cancer cells was significantly decreased after depletion of either CD44 or HA. In vivo, they found that CD44 deletion in breast cancer cells resulted in a delay in tumor formation and localized progression. This finding was accompanied by a decrease in infiltrating CD206+ macrophages, which are typically associated with tumor promoting functions (Witschen et al. 2020). Furthermore, a recent study has shown the importance of altering HA metabolism to malignant progression of breast carcinoma. Arnold et al. proved that increased HA synthesis requires reprogramming of the glucose metabolism and that disrupting of this metabolic reprogramming through the genetic and pharmacological depletion of the HA precursor UDP-glucuronic acid significantly reduces cellular invasion and colony formation in vitro in MDA-231 cells. Even more, results of in vivo mesenchymal-like breast cancer models revealed a reduction in tumor growth and metastasis (Arnold et al. 2020).

These data suggest that HA plays a key role in proliferation, invasion, and chemoresistance events in breast cancer and that it does so through different mechanisms.

7.3.1.2 HA and Ovarian Cancer

Epithelial ovarian cancer (EOC) is the commonest cause of gynecological cancer-associated death and the fourth cancer-related deaths in women in the developed world (Jayson et al. 2014). Approximately 90% of all ovarian cancers are epithelial

and of these ~80% represent the serous histological subtype (Hiltunen et al. 2002). Conventional ovarian cancer treatment comprises first-line chemotherapy with a carboplatin-paclitaxel regimen and cytoreductive surgery (Marchetti et al. 2010; Vaughan et al. 2011). However, although this regimen is initially effective, there is a high recurrence rate and most ovarian cancer patients suffer at least one relapse within 12–18 months (Colombo et al. 2006, 2017; Kartal-Yandim et al. 2016; Yousefi et al. 2016) owing to chemoresistance and metastasis (Lengyel 2010; Amoroso et al. 2017). Increased expression of HA has been shown to be closely correlated with the epithelial ovarian cancer (EOC) degree and metastatic potential (Anttila et al. 2000; Ween et al. 2011), with a 100-fold increase in HA expression in grade three EOC (Hiltunen et al. 2002). Different antecedents suggest that targeting the synthesis or accumulation of HA represents a promising therapeutic approach for the treatment of ovarian cancer. Lokman et al. showed that inhibition of HA production by 4-MU in combination with chemotherapy with carboplatin (CBP) significantly reduced chemo-resistant serous ovarian cancer cells survival and spheroid formation and increased apoptosis of these cells compared to CBP alone. Even more, showed that 4-MU alone or combined with CBP significantly decreased the invasion ability of these cells *in vivo* compared to control treatment. The fact that the inhibitor of the synthesis of HA 4-MU allows to overcome the chemoresistance to the chemotherapeutic drug CBP and that they determined that the production of HA increases in ovarian cancers resistant to chemotherapy indicates that the inhibition of HA is, therefore, a promising new strategy to overcome chemoresistance and improve ovarian cancer survival (Lokman et al. 2019). Even more, the reduction of HA-pericellular coat was related with the inhibition of cell migration, proliferation, and invasion (Anttila et al. 2000). On the other hand, clear-cell ovarian cancers, an epithelial ovarian cancer subtype, show a spherule-like mucoid stroma with a hollow acellular space. Despite the absence of stromal cells, both the mucoid stroma and hollow spheroids contain abundant ECM, mainly composed of HA that plays a crucial role in the formation of that structures and tumor progression. In this sense, Kato et al. showed that when HA synthesis was inhibited by 4-MU in HAC-2, a clear cell carcinoma cell line, the spherule-like accumulation of HA, or hollow spheroids were not observed. Moreover, the inhibition of HA synthesis was associated with the reduction of cell growth (Kato et al. 2016). Even more, Lin et al. found that upregulation of UDP-glucose dehydrogenase (UGDH) is related to ovarian cancer metastasis and that knockdown of UGDH significantly decreased wound healing and migration and proliferation in three ovarian cancer cell models, TOV21G, A2780, and HeyA8 cells. Furthermore, in an *in vivo* xenograft model from TOV21GHI cells, they demonstrated that UGDH removal significantly decreased ovarian cancer tumor growth (Lin et al. 2020). Given that the UDP-glucose dehydrogenase converts UDP-glucose to UDP-glucuronic acid, a precursor of several GAGs and PGs presents in the ECM, as well as cell proliferation and migration, it is likely that the elimination of UGDH decreased HA levels in ovarian cancer and thus affected the metastatic ability of ovarian cancer. However, further studies are needed to demonstrate the biological effects of HA from ECM in ovarian cancer.

7.3.1.3 HA and Prostate Cancer

Patients with clinically localized prostate cancer (CaP) are often treated with radical prostatectomy or radiation with curative intent. However, the disease recurs in a substantial number of the patients and becomes hormone refractory.

Lipponen et al. found high levels of HA in the stroma of prostate cancer and showed that stromal HA accumulation is related to poor prognosis in prostate cancer (Lipponen et al. 2001). Posey et al. showed that in radical prostatectomy specimens, HA expression is elevated in CaP tissues, but it is not an independent predictor of biochemical recurrence (Posey et al. 2003), besides has been observed that HA and HYAL-1 expression in prostate biopsy specimens and correlated in with disease recurrence (Gomez et al. 2009). Lokeshwar et al. studied the effects of inhibition of HA synthesis by 4-MU on five prostate cancer cell lines, LNCaP, DU145, PC3-ML, LAPC-4, and C4-2B. They demonstrated that 4-MU inhibited the proliferation of all cell lines by inducing apoptosis, in a dose-dependent manner, and that this effect of 4-MU on cell growth and apoptosis was due to the inhibition of HA synthesis. Even more, they demonstrated that 4-MU inhibited invasive activity and chemotactic motility of PC3-ML and DU145 cells by inhibiting HA production. In PC3-ML xenograft mice model, oral administration of 4-MU significantly inhibited tumor growth and reduced microvessel density in tumors from 4-MU-treated animals (Lokeshwar et al. 2010). In a transgenic adenocarcinoma of the prostate model, oral administration of 4-MU also significantly decreased microvessel density and prostate cancer cells proliferative index. Furthermore, daily gavage of 4-MU inhibited tumor growth in a DU145 subcutaneous xenograft model and inhibited skeletal metastasis in the jaw, pelvis, femur, and spinal cord in a PC3-ML intracardiac bone metastasis model. Even more, they showed that all these effects were the result of inhibition HA synthesis and signaling (Yates et al. 2015). Different studies showed that, in addition to the accumulation of HA in the tumor stroma, the alteration of hyaluronic acid synthase and hyaluronidase in tumor epithelial cells are associated with increased cell proliferation, invasion, metastasis, and poor outcome in men who have undergone radical prostatectomy (Simpson and Lokeshwar 2008; Bharadwaj et al. 2009).

7.3.1.4 HA and Pancreatic Cancer

Pancreatic cancer is one of the most difficult conditions to treat, although it only accounts for 4% of all cancers; 5-year survival is less than 10% in patients with the disease and has remained relatively unchanged over the past 25 years (Siegel et al. 2016). Most patients present with locally advanced or metastatic disease, and such individuals have a grim median survival of 6–10 months, and 3–6 months, respectively. Although 10–15% of patients have potentially resectable tumors, the recurrence rate of the disease following surgery is high (Wong and Lemoine 2009). The

vast majority (90%) of pancreatic cancers (PC) are tumors originating from pancreatic ductal cells (Sener et al. 1999; Sharma et al. 2011).

The most common subtype of human pancreatic malignancies is Pancreatic Adenocarcinoma (PDAC). This type of cancer originates in the ductal epithelium and evolves from premalignant lesions to fully invasive cancer. It has been established that PDAC is preceded by the evolution of precursor lesions called pancreatic intraepithelial neoplasia (PanIN 1A/B, 2, and 3) (Hruban et al. 2001), and, under certain conditions, Acinar Ductal Metaplasia (ADM) might be critical for the development of PanIN lesions (Means et al. 2005). The progression from histologically normal ductal epithelium to low-grade PanIN to high-grade PanIN is associated with the accumulation of specific genetic changes (Guerra et al. 2007; Hidalgo 2010). It has also been reported that elevated expression of autophagy in cancer cells has been implicated in the development of PDAC (Rui Kang 2012).

PDAC is characterized by a dense stromal matrix also referred to as TME. This stromal matrix starts to evolve early around PanIN lesions. Moreover, in established carcinomas, the TME constitutes up to 90% of the tumor mass. PDAC TME is composed by various cellular and acellular components. Between the acellular components, large amounts of collagen, fibronectin, proteoglycans, and HA are present (Provenzano et al. 2012; Jacobetz et al. 2013). Particularly, HA is consistently increased in pancreatic cancer stroma, where exerts a pro-tumoral action (Sato et al. 2016), including tumor growth (Toole and Hascall 2002), cell proliferation, invasion (Cheng et al. 2016), and metastasis (Zhang et al. 1995; Toole 2002). These actions, associated with tumor progression, require that HA binds to its specific receptors. The main binding receptor, CD44 is located on the cell surface, and the intracellular RHAMM (receptor for hyaluronan-mediated motility) in the cytoplasm, where it activates the PI3K/Akt and ERK1/2 signaling pathways (Zhu et al. 2013; Lokeshwar et al. 2014). Moreover, CD44 has been recognized as a cancer stem cell marker of PDAC, involved in both epithelial mesenchymal transition and multidrug resistance; mechanisms involved in cancer cells protection from chemotherapeutic agents (Zhang et al. 2012; Wei et al. 2013). The secretion of HA by tumor cells facilitates the action of cancer-associated fibroblast. These cells produce growth factors, hormones, and cytokines to stimulate HA production by PDAC cells, which stimulates and promote their malignant behavior. As a result, the interaction between HA and its receptors provides tumor cells the suitable microenvironment to survive, proliferate, and invade. HA not only works as a tumor promoting factor, but also provides a barrier to the arrival of chemotherapeutic agents. In this sense, the accumulation of HA in TME increases the interstitial fluid pressure (IFP), which makes drug perfusion very difficult (Dufort et al. 2016a, b). It was described that hyaluronidase treatment can rapidly reduce the IFP in a mouse model of PDAC and significantly improve the appropriated response to gemcitabine (Provenzano et al. 2012). It is correct to think that reducing the level of HA in the ECM may offer an effective approach to treating PDAC.

It is attractive to presume that the inhibition of HAS individually with specific antibodies or the use of promoters of each hyaluronidase would be an adequate strategy to inhibit tumor progression and induce remission. However, the expression

of these enzymes in pancreatic cancer cells is heterogeneous (Cheng et al. 2016), suggesting that this strategy could not result in a successful response in most patients with PDAC. Rather, the use of compounds that reduce the synthesis of HA could be suitable as a strategy of HA reduction. Among them, 4-methylumbelliferone (4-MU), which stably suppresses the synthesis of HA *in vitro* and *in vivo*, has been widely accepted because of its mechanism of action and because it is innocuous to organisms (Kudo et al. 2017). Two therapeutic strategies were proposed: the combination of 4-MU with gemcitabine, the first-line chemotherapeutic agent used for decades, and decombination of 4-MU with immunotherapy. For the first option, it was described that 4-MU inhibited the formation enveloped HA-rich matrix, promoting the perfusion of gemcitabine (Nakazawa et al. 2006). In another study, in a xenograft mouse model, electron microscopic observation revealed that 4-MU reduced the amount of HA in tumors surrounding, on the backs of animals and altered the intercellular space (Yoshida et al. 2016). Moreover, 4-MU orally administered, enhanced the survival rate of mice that were intraperitoneally inoculated with human PDAC cells. In these mice, intratumoral HA level was reduced in a 30% compared to control mice (Yoshida et al. 2016). This result is really encouraging, because it is supposed that orally administered 4-MU is distributed throughout the whole body, assuming that the beneficial effect would be not only on the primary tumor but also on distant metastases, causing suppression of proliferation, migration, and invasion activities.

The other therapeutic strategy involves the combination of 4-MU and immunotherapy. Within the last, immune checkpoint inhibitor therapy (targeting CTLA4, PD1, PDL1) or the perfusion of immune cells have not been remarkably successful against PDAC probably because of the strong and extensive ECM rich in HA. In this context, HA plays an integral role in this immune evasion. As explained above, the reduction of HA in the tumor ECM reduces the IFP, promoting drug perfusion as well as the arrival of immune cells. It was described that the administration *in vivo* of 4-MU reduced intratumoral HA level and promoted infiltration of inoculated $\gamma\delta$ T-cells into tumor tissue, with the consequent suppression of tumor growth (Suto et al. 2019). However, further research is needed to achieve an adequate potential combined therapy. But it is promising that the control of HA will become an adjuvant treatment that can be used in combination with different immunotherapies, enhancing their efficacy through the remodeling of the ECM around cancer cells.

7.3.1.5 HA and Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cancer cause of death globally (Brenner et al. 2014). The global burden of CRC is projected to increase by 60% within 2030, especially in countries with medium and high human development index (HDI) scores (Wong et al. 2021). CRC originate primarily from genetic and epigenetic alterations in epithelial cells, leading to the development of benign polypoidosis, develops into adenoma and then carcinoma,

and finally the invasion of the interstitial matrix. Those processes require the degradation and modification of the existing ECM (Onfroy-Roy et al. 2020).

Several studies have shown the importance of HA in this type of cancer. HA levels are higher in cancer tissues than in noncancerous tissues. The stromal tissue stains highly intensely and the tumor epithelium is mostly HA negative, and this feature is associated with tumor grade, thus high-grade tumors have strong HA intensity. Furthermore, patients with strong HA intensity in tumor epithelium have a low survival rate (Wang et al. 1996; Ropponen et al. 1998). Also, cytosolic HA levels are correlated with clinical tumor stage, thus, patients with more advanced tumors have higher HA levels. However, HA content is not correlated with age, sex, tumor location, or histological grade (Llaneza et al. 2000). Zhang et al. evaluated the molecular weight (MW) of abnormally elevated HA in cancer tissues. HA MW was approximately 6 kDa and it was positively correlated with metastasis and invasion of CRC. Besides, 6-kDa HA was found elevated in sera from CRC patients and they observed a significantly positive correlation between 6-kDa HA expression in primary CRC lesions and matched serum samples from these patients (Zhang et al. 2019).

HA binds to CD44 that is expressed on the plasma membrane of many cells but also binds to other receptors including toll like receptor 4 (TLR4) that is widely distributed in the gastrointestinal tract. HA binding to TLR4 promotes epithelial repair in the DSS-colitis and radiation injury models (Riehl et al. 2012) and mediates the normal growth of the intestine and colon including the proliferation of stem cells (Riehl et al. 2015). However, HA-CD44-TLR4 interactions are involved in pathogenesis of CRC (Kim et al. 2004). Makkar et al. demonstrated that HA binding to TLR4 and CD44 promotes cell proliferation and blocks spontaneous apoptosis. In addition, TLR4 and CD44 knockdown or HA blocking by PEP1 slows tumor growth and reduces tumor volume (Makkar et al. 2019). Even more, the decrease in GAGs synthesis due to UDP-glucose dehydrogenase (UGDH) downregulation and 4-MU treatment reduce CRC cells growth and motility, indicating that invasive ability of CRC cells is HA dependent (Wang et al. 2010).

HA synthases (HAS) are also involved in the development and progression of CRC. The expression of HAS3 was known to produce smaller HA fragments with molecular size between 50 and 1000 kDa, as well as total HA production were found to be increased in metastatic CRC cells when compared with cells isolated from a primary tumor. Furthermore, downregulation of HAS3 in a highly tumorigenic CRC cell line decreased subcutaneous colon cancer growth in a mouse model by increasing apoptosis rate of these cells (Bullard et al. 2003). Even more, Kim observed a correlation between HAS2 levels and malignant phenotypes of CRC cells. HAS2 expression was significantly induced in CRC tissue samples and CRC cell lines. Furthermore, HAS2 depletion increased apoptosis, therapeutic sensitivity, and decreased epithelial to mesenchymal transition-related migration and invasive ability of CRC cells (Kim et al. 2019).

HA may influence metastasis of colon carcinoma cells. Laurich et al. demonstrated that metastatic colon carcinoma cells that express high levels of HAS and

synthesize large pericellular HA matrices adhere more avidly to laminin than cells isolated from a primary tumor that secrete and retain less HA (Laurich et al. 2004).

7.3.1.6 HA and Lung Cancer

Lung cancer is the leading cause of cancer death around the world. It arises from the cells of the respiratory epithelium and can be classified into two categories. Small cell lung cancer (SCLC) is a highly malignant tumor derived from cells exhibiting neuroendocrine characteristics and accounts for 15% of lung cancer cases and non-small cell lung cancer (NSCLC), which accounts for the remaining 85% of cases (dela Cruz et al. 2011). The pulmonary ECM provides mechanical stability and elasticity, which are essential for physiological lung function during the breathing, inhalation, and exhalation. Lung-ECM is mainly composed of collagens, proteoglycans, and glycoproteins and serves as a reservoir for several growth factors and cytokines, which are crucial for cell differentiation and proliferation. Lung tumors are surrounded by an extensive stroma and interactions between cancer cells-ECM protects cells from chemotherapy-induced apoptosis (Burgstaller et al. 2017).

The content of GAGs in cancer tissues is higher compared to normal tissues and the amount of these polysaccharides is different in every histologic type (Horai et al. 1981). HA concentration is increased mainly in squamous cell carcinoma, and it is related to the low survival of patients (Pirinen et al. 2001; Rangel et al. 2015). Moreover, sputum from lung cancer patients has higher HA concentration levels compared to cancer-free and healthy people (Rangel et al. 2015). Also, HA receptors are related to the development of lung cancer. RHAMM is upregulated in non-small cell lung carcinomas, especially in metastatic tumors (Wang et al. 2016) and there is an increase in LYVE-1 levels in tumor tissue and periphery related to lymph nodes metastasis (Renyi-Vamos et al. 2005).

HA is produced by tumor cells and stroma cells and several studies have shown the importance of HA in the progression of lung cancer. Song et al. demonstrated that conditioned medium of NSCLC cells increases HA production by lung cancer-associated fibroblasts (LCAFs) and the inhibition of HA production by these cells reduces tumor cells growth and proliferation. Even more, simultaneous silencing of HAS2 and HAS3 and CD44/RHAMM silencing reduced HA production and in vitro proliferation of NSCLC cells (Song et al. 2019).

On the other hand, HA production is regulated by cytokines and growth factors like tumor necrosis factor alpha (TNF- α), tumor growth factor beta (TGF- β), and endothelial growth factor (EGF), produced by tumor cells, fibroblasts, and immune cells (Tammi et al. 2011). Mulshine et al. demonstrated that NSCLC cells treatment with TGF/ β enhance HA production by malignant cells and this increase is related to epithelial to mesenchymal-like transition in NSCLC cells (Mulshine et al. 2010).

7.3.2 *HA and Sarcomas: Osteosarcoma, Chondrosarcoma, and Fibrosarcoma*

Osteosarcoma (OS) is the most common primary bone tumor and responsible for considerable morbidity and mortality due to its high rates of pulmonary metastasis. The prognosis of OS patients has improved dramatically with the introduction of chemotherapy; however, cases with metastases or unresectable tumors still have a poor prognosis (Arai et al. 2011). Several studies have implicated HA-rich ECM on osteosarcoma progression (Nishida et al. 2005; Tofuku et al. 2006; Hosono et al. 2007). Tofuku et al. found that HA synthesized by HAS3 promoted proliferation, invasion and degradation of ECM, biological functions crucial for metastasis and showed that the inhibition of HA synthesis by 4-MU suppressed proliferation and invasion of LM8 cells in vitro (Tofuku et al. 2006). Nishida et al. showed that the selective inhibition of HAS-2 mRNA in MG-63 osteosarcoma cell line by antisense phosphorothioate oligonucleotides reduced HA production by these cells what disrupted the assembly of cell-associated matrices and, consequently, hindered cell proliferation, motility, and invasiveness (Nishida et al. 2005). In a later study, these same authors examined the effects of exogenously added HA oligosaccharides on tumorigenicity of murine osteosarcoma cells LM-8, highly metastatic in mice, and human osteoblastic osteosarcoma cells, MG-63 and proposed that these small oligosaccharides compete with the binding of high-molecular-mass HA for cell surface receptors such as CD44 as a mechanism to deplete HA-rich matrices from cells. They determined that the treatment of MG-63, which have abundant HA-rich cell-associated matrix, as well as LM-8, with HA oligos inhibited the formation of HA-rich cell-associated matrix, resulting in the inhibition of growth, motility, and invasiveness and induction of apoptotic activity in vitro. Even more, daily application of HA oligosaccharides showed a trend toward inhibition of LM-8 tumor growth in vivo. These data suggest that the abrogation of hyaluronan-rich cell-associated matrices have potent antitumor effects (Hosono et al. 2007). Similarly, Arai et al. showed that the inhibition of HA retention by 4-MU reduced the formation of functional cell-associated matrices in OS cells, and inhibited cell proliferation, migration, and invasion, resulting in the reduction of tumorigenicity and lung metastasis. Even more, although 4-MU showed only a mild inhibitory effect on the growth of the primary tumor in vivo, it markedly inhibited the development of lung metastasis by inhibiting HA retention (Arai et al. 2011). Even more, Gvozdenovic et al. showed that overexpression of CD44 in SaOS-2 cells enhances intratibial primary tumor growth and formation of pulmonary metastases using an intratibial xenograft OS mouse model. These malignancy-enhancing effect of CD44s was HA-dependent and was reflected in the significant increase of primary tumor volume and the numbers of pulmonary micro- and macrometastases when compared to that of control SaOS-2 cells. These results suggest that CD44s/HA interactions play a key role in OS (Gvozdenovic et al. 2013). Even more, it was shown that hyaluronidase increased the transcapillary pressure gradient in OS xenografts by degrading extracellular HA and remodeling ECM, thus improving the uptake and

distribution of liposomal doxorubicin (Eikenes et al. 2005). Taken together, these reports highlight the involvement of HA in the progression and metastasis of OS.

Chondrosarcoma, the second most common primary malignant bone tumor, particularly when low-grade, is also characterized by the formation of a HA-rich ECM which has been considered as one explanation of drug resistance (van Oosterwijk et al. 2012). In this sense, Hamada et al. showed that the inhibition of HA synthesis by 4-MU suppressed cell proliferation, migration, and invasiveness of chondrosarcoma cells. Even more, in an *in vivo* model of chondrosarcoma, the inhibition of HA production markedly inhibited grafted tumor growth (Hamada et al. 2018). Recently, Koike et al. evaluated the effects of a novel hyaluronidase, KIAA1199, on ECM formation as well as antitumor effects on chondrosarcoma. They demonstrated that the KIAA1199 forced expression did not affect proliferation or apoptosis but inhibited migration and invasion of RCS cells *in vitro*. In contrast, the expression of KIAA1199 significantly inhibited the growth of grafted tumors. Although there was no direct inhibitory effect on proliferation *in vitro*, induction of KIAA1199 showed the antitumor effects in grafted tumor growth *in vivo* possibly due to changes in the TME such as inhibition of ECM formation. Together, KIAA1199 could be a novel promising therapeutic tool for low-grade chondrosarcoma, mediated by the degradation of HA (Koike et al. 2020).

Other antecedents have also provided direct evidence for the role of HA in tumorigenicity in fibrosarcoma, another of the most common sarcomas. In this case, Kosaki et al. showed that overproduction of HA by expression of the HAS2 enhances growth of tumors in xenograft models (Kosaki et al. 1999).

7.3.3 *HA and Glioblastoma*

Glioblastoma (GBM), also known as grade IV astrocytoma by the World Health Organization classification (Louis et al. 2016), is the most frequent primary tumor of the central nervous system (CNS) in adults. It is characterized by fast growth, invasiveness, and high mortality, with a median survival of less than 15 months after diagnosis (Anjum et al. 2017). Currently, there are few therapeutic options (le Rhun et al. 2019; Perus et al. 2019; Strobel et al. 2019), with the first-line therapy being surgical resection and radiotherapy combined with cycles of temozolomide (TMZ). Unfortunately, TMZ therapy causes severe adverse effects, and almost 50% of patients exhibit resistance to the treatment (Anjum et al. 2017; Philteos et al. 2019; Strobel et al. 2019). Furthermore, high inter- and intratumor heterogeneity, individual variability and different stages of disease at diagnosis time complicate GBM treatment (Rajaratnam et al. 2020).

For several years there is a growing interest in studying the CNS's ECM and the mechanisms through which it impacts in the development and progression of brain tumors, with obvious implications for the development of new therapeutic alternatives. In the CNS, HA is the main component of ECM, beside with proteoglycans (such as aggrecan, versican, neurocan, and brevican without collagen), the tenascins

and link proteins, and other GAGs, including chondroitin sulfate, heparan sulfate, and keratan sulfate (Eikenes et al. 2005). In the CNS, HA participates in the correct generation, proliferation, and maturation of neural stem cell progenitors (NSCP) during brain development and repair (Su et al. 2019). Reports published in the 70s and 80s established that HA has another, undesirable role: these studies demonstrated that production of GAGs—and particularly HA—by malignant glioma cells was higher than those in normal glial cell lines (Pibuel et al. 2021b), and which was associated with a higher rate of cell proliferation and several publications did confirm a correlation between the addition of HA and an enhanced rate of invasion across multiple glioma cell lines (Chintala et al. 1996; Nakagawa et al. 1996; Radotra and McCormick 1997; Pibuel et al. 2021a).

After that, several approaches have been used in the study of the relationship between HA and GBM including *in vitro* 2D and 3D assays, *in vivo* models and patient samples (Pibuel et al. 2021b). In GBM, CD44 is strongly involved in cell invasion (Mooney et al. 2016), and it was demonstrated that optimal levels of this receptor were necessary for GBM cells to generate highly infiltrative tumors in a mouse model (Klank et al. 2017), while the treatment with an anti-CD44 monoclonal antibody inhibited tumor growth of local glioma (Breyer et al. 2000). Regarding RHAMM, it has been associated with an increase in migration and proliferation of glioma cells, and its levels have been correlated with tumor grade (Akiyama et al. 2001; Virga et al. 2017; Lim et al. 2017). Although the importance of CD44 and RHAMM in mediating HA's functions is clear, other receptors such as EGFR in association with the HA receptors could explain some of the effects of HA on GBM progression. It was demonstrated that CD44 through the bind of EGFR form complex, that might provide a mechanism for HA-mediated cell invasion and proliferation (Tsatas et al. 2002).

After these interactions, HA activates several signaling pathways being the most relevant MEK/ERK, c-MYC, PI3K/Akt, FAK, RhoA/ROK, and the transcription factor NF- κ B (Pibuel et al. 2021b), enhancing all the malignant features of GBM, mostly proliferation, migration, and invasion but also chemoresistance (Kim et al. 2005). The activation of these signaling pathways increases the activity of MMPs, the CD44 cleavage and in turn an increase of migration and invasion of this tumor type (Chetty et al. 2012).

Regarding the tissue samples of patients, glioma lesion showed HA content much higher than in adult normal brains (Delpech et al. 1993); however, the proportion of HA was inversely associated with glioma grade (Sadeghi et al. 2003). Interestingly, although HA failed as a molecular marker, HAS2 and HYAL-2 were associated with tumor grade and even correlate with patient outcomes (Valkonen et al. 2018; Pibuel et al. 2021b).

Considering the poor outcomes of the patients with GBM, several studies focused its efforts on the development of new therapeutic alternatives and molecular targets. The increases of HA in tumor tissues compared to the healthy brain, discussed above, may not have obvious clear cut diagnostic potential, but they do implicate HA in GBM progression, the results suggest its potential as a therapeutic target. HA

degradation has been explored as a therapeutic target in several studies, and some clinical trials have shown that treatment with HYAL as adjuvant chemotherapy contributes to clinically relevant remissions in high-grade astrocytoma and to the efficacy of chemotherapy in pediatric brain tumors (Baumgartner et al. 1998; Martinez-Quintanilla et al. 2015). In addition, it was recently reported that treatment with HYAL, alone or in combination with TMZ, showed a cytotoxic effect on the GSC population (Hartheimer et al. 2019). Interesting results were also obtained with oHA. The oHA treatment improved the effects of both radiation and methotrexate and even TMZ on GBM cells (Maria et al. 2008; Karbownik et al. 2014). Finally, as a new alternative, the use of 4MU showed a marked effect on GBM cells decreasing metabolic activity, cell proliferation, migration, and MMP-2 activity while causing high levels of apoptosis in murine GL26 GBM cells. Moreover, our group showed that 4MU partially diminishes HA synthesis, although several of its effects would be independent of this inhibition (Pibuel et al. 2021a).

Due to this, it is one of the main targets for the investigation is to find alternative therapies that seek to control tumor spread and reduce mortality, one way is understanding the interaction of HA with GBM cells.

In this sense, the mechanical interplay between the recruited cell stroma, the ECM, and the tumor cells has therefore attracted significant attention as a new avenue of therapy, termed stromal therapy.

7.4 Conclusion and Perspectives

To sum up, we could conclude that the ECM component GAG molecule, hyaluronan has tumor specific characteristics, and is an important factor in the development and progression in different hematological and solid tumors. It is involved either in direct or indirect way in all process defined as the hallmarks of cancer (Fig. 7.2).

It is possible to conclude that, molecules involved in HA metabolism and signals could be considered as clinical targets for tumors beyond consideration of the etiology. However, for the clinical application, it is important to consider that the impact of its modulation effect depends on the type of tumor. Thus, its application could be associated with induction of tumor cell death, avoidance of drug resistance to the enhancement of immunotherapy. With this perspective, we have described and discussed the different aspects of the role of HA in the biology and therapy of various tumors in this chapter. Finally, we aimed to support help the strategies for pharmacological application able modulate HA and consequently different processes in cancer progression.

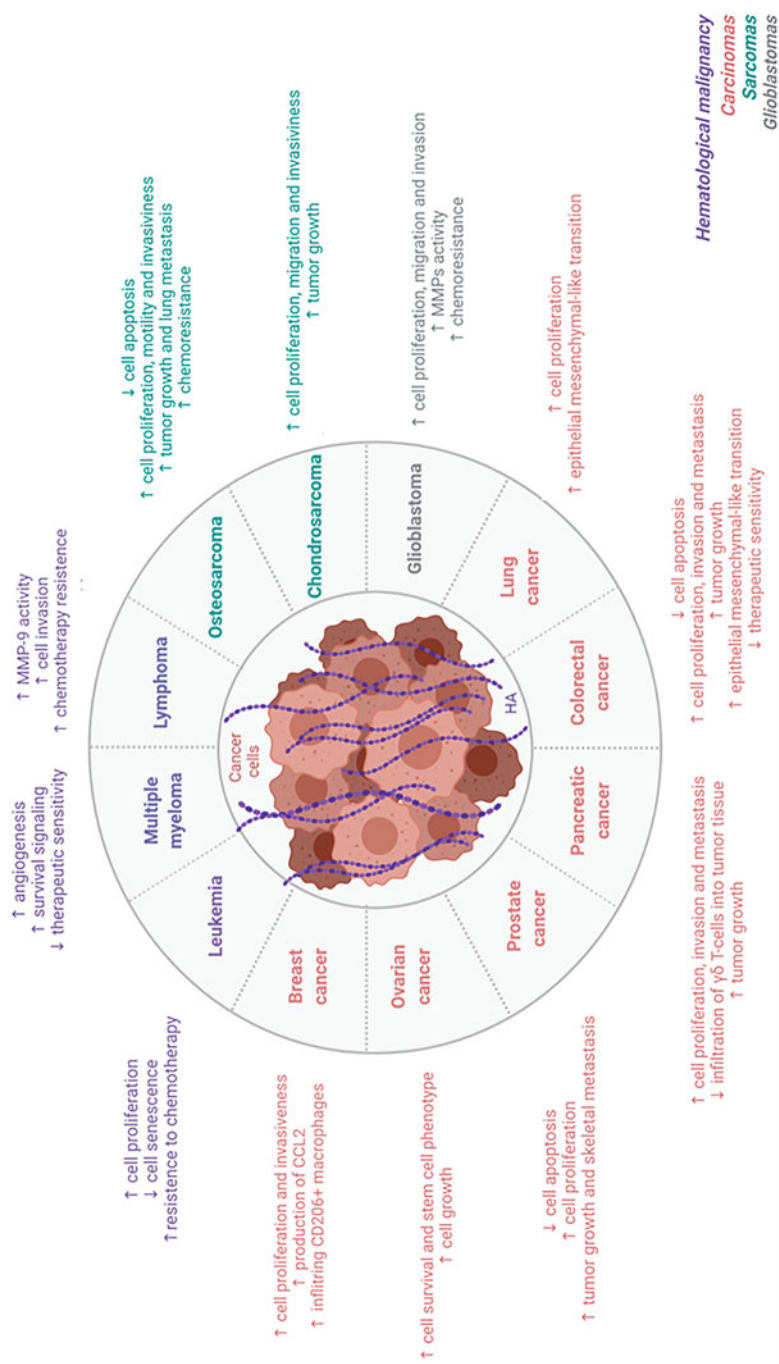


Fig. 7.2 Biological effects of ECM Hyaluronan (HA) in hematological malignancy and in carcinomas, sarcomas and gliomas

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