Review

HA in pathological and physiological processes: An overview of HA metabolism in cancer and pregnancy

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ABSTRACT

Hyaluronan (HA) is a glycosaminoglycan component of the extracellular matrix, where it possesses functions in morphogenesis, tissue injury and repair, inflammation, and tumorigenesis. HA is synthesized by HA synthases (HAS) and is degraded by hyaluronidases (HYAL). Increased levels of HA in tumors, due to differential expression of HAS and HYAL, are related to tumor progression and metastasis. During pregnancy, changes in HA deposition and distribution indicate that HA participates in preparation of the endometrial stroma for reception of the embryo. While pregnancy is a physiological state and cancer a complex and unpredictable pathology, both trophoblast and tumor cells share some proliferative, invasive, and immune tolerance mechanisms that allow establishment of pregnancy and tumor progression. This indicates that the study of a physiological condition such as pregnancy could help to find novel cancer treatment strategies. Abnormal HA metabolism has been observed in cancer and pathologies of pregnancy, thus indicating that HA could be considered a key molecule, able to regulate these processes. This review discusses the role of HA in cancer and pregnancy: the study of similarities and differences between both models could lead to identification of new targets for treatment of tumors or pregnancy-associated pathologies.

KEYWORDS: hyaluronan, cancer, pregnancy, hyaluronan synthase, hyaluronidase

ABBREVIATIONS

4-MU, 4-Methylumbelliferone; Da, Dalton; DC, dendritic cells; ECM, extracellular matrix; GAG, glycosaminoglycan; GlcA, D-glucuronic acid; GlcNAc, N-acetyl-D-glucosamine; HA, hyaluronan; HAS, HA synthase; HYAL, hyaluronidase; HMW-HA, high molecular weight-HA; LMW-HA, low molecular weight-HA; oHA, HA oligosaccharides

INTRODUCTION

Hyaluronan (HA) is a linear glycosaminoglycan (GAG), composed of repeated disaccharide units of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc) [1]. HA is a conspicuous component of the extracellular matrix (ECM), where it possesses several functions both in

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physiological and pathological conditions such as morphogenesis, tissue repair, inflammation and tumorigenesis [2-5]. Upon interaction with cell surface receptor CD44, receptor for hyaluronic acid-mediated motility (RHAMM), Toll-like receptor 4 (TLR-4) or HA binding-proteins (TSG-6, SHAP), HA modulates fundamental cell behaviors such as cell proliferation, apoptosis, adhesion, migration and differentiation [3]. In healthy tissues, HA is present as a native molecule of high molecular weight (HMW-HA) up to 10^6-10^7 Da, which has a structural function influencing hydration and physical properties of tissues. However, during inflammation it is also found as a molecule of low molecular weight (LMW-HA) ranging from $1x10^4$ to $1x10^6$ Da, or small fragments usually called hyaluronan oligosaccharides (oHA) which are generated by enzymatic or hydrolytic digestion. Several evidences suggest that degraded forms of HA are present in tumors and inflammation and have distinct function than native HA [6, 7]. Thus, molecular weight and concentration of HA are crucial for its function [8, 9].

Tumor growth is a very complex process involving cell proliferation and the establishment of an invasive phenotype, as well as acquisition of a rich blood supply and evasion of the immune system. These mechanisms are highly dependent on interactions between tumor cells and their surrounding microenvironment, which is composed of stromal and immune cells, ECM components, growth factors and cytokines [10, 11]. HA is a component of the ECM surrounding tumors highly implicated in human tumor progression and metastasis [12, 13], due to an imbalance between HA synthesis by hyaluronan synthases (HAS) and its degradation by hyaluronidases (HYAL).

Pregnancy is a unique biological event characterized by delicate interactions between the conceptus and the maternal environment. Development of the embryo to the blastocyst stage, its adhesion to the endometrium and subsequent invasion of the underlying stroma leading to formation of placenta are the result of complex mechanisms that involve endometrial modifications and maternal immunological tolerance [14]. The remodeling of the endometrial architecture is essential for creating a suitable environment for blastocyst implantation. This process includes phases of cellular proliferation, differentiation and tissue breakdown along with alterations in the composition of ECM [15, 16]. During gestation, changes in HA deposition and distribution indicate that HA participates in preparation of the endometrial stroma for embryo implantation [17, 18].

While pregnancy is a physiological state characterized by controlled cell proliferation, invasion, angiogenesis and immune evasion and cancer is a complex and unpredictable pathology, both trophoblasts and tumor cells share some proliferative, invasive, and immune tolerance mechanisms that allow establishment of pregnancy and tumor progression [19]. Thus, studying a physiological condition could help to find novel cancer treatment strategies. On the other hand, studying factors involved in cancer progression might help to understand aspects in abnormal pregnancy. As a common component of the tumor and uterine microenvironment and due to its variety of biological functions, HA could have important implications in the modulation of tumor growth and embryo implantation and represent an appealing target for the development of therapeutic strategies. This review presents current evidence on the role of HA in both tumorigenesis and pregnancy, with special emphasis in the regulation of HA metabolism and its relationship with cancer and adverse pregnancy outcomes.

Hyaluronan synthesis and degradation

HA biosynthesis is carried out at the inner face of the plasma membrane by hyaluronan synthases (HAS) (Figure 1) and the growing polymer is extruded through the membrane into the extracellular space [20]. There are three HAS isozymes (HAS-1, HAS-2 and HAS-3) that catalyze the addition of GlcA and GlcNAc to the growing HA polymer [21, 22]. Among HAS, HAS-2 is crucial to embryonic development since its deletion produces cardiac and vascular abnormalities and mice dye at gestation day 9.5 [23]. The main differences between these isozymes are the catalytic activity and the size of the synthesized HA molecules. It has been demonstrated that HAS-1 and HAS-2 produce

predominantly HMW-HA, whereas HAS-3 synthesizes HA of lower molecular mass [24]. HAS-2 is commonly expressed in mammalian tissues, whereas HAS-3 is expressed under specific conditions such as tumorigenesis or inflammation. However, the expression profiles of HAS genes are differentially regulated during physiological and pathological conditions and differences observed during these processes can be explained, at least in part, by the signaling pathways that are triggered by various growth factors and cytokines. Among them, the epidermal growth factor activates HAS-2 in keratinocytes [25] and the platelet-derived growth factor BB (PDGF-BB) also induces HAS-2 in mesothelial cells [26] and fibroblasts [27]. On the other hand, transforming growth factor-β (TGF-β) increases HAS-1 expression in synovial fibroblasts [28], while in mesothelial cells it decreases HAS-2 expression [29]. These findings indicate that diverse cell types respond differently to modulators of HA synthesis. HAS activity can be also regulated at the post-translational level by phosphorylation [30, 31], dimerization and ubiquitination [32]. Besides, it has been also observed that cellular UDP-N-acetylhexamine content regulates HA synthesis [33, 34].

Degradation of HA in tissues takes place both *in situ* and by release into the lymph and vascular systems. In humans, 5 g of the 15 g of total body HA turnover daily, mostly by the action of the HYAL enzymes [35]. The HA that is not locally metabolized in the tissues enters the lymph system and is removed mainly by the lymph nodes, whereas the HA in the blood stream is removed mainly by the liver endothelial cells. Besides spleen and kidney can also uptake HA [1]. The uptake of HA from lymph or circulation is mediated mainly by receptors such as HA receptor for endocytosis (HARE) [36]. It has been estimated that the half-life of HA in serum is of minutes, in the skin is about 1 day, in cartilage 1-3 weeks and in the vitreous humour 70 days [1]. Once inside the cells, HA degradation occurs by the concerted action of three specific enzymes: a hyaluronidase (HYAL) and two exoglycosidases (β-glucuronidase and β-N-acetyl hexosaminidase). Hyaluronidases are endo-β-N-acetyl hexosaminidases that cleave β-1-4-glycosidic bonds and generate

HA fragments that are digested by exoglycosidases, which remove the terminal sugar residue and generate GlcA and GlcNAc [37]. In humans, six hyaluronidase genes have been identified. These are HYAL-1, HYAL-2, HYAL-3, HYAL-4, SPAM1 and PHYAL1, which respectively encode HYAL-1, HYAL-2, HYAL-3, HYAL-4, PH-20 and a non-translated pseudogene [38]. Among these enzymes, HYAL-1 and HYAL-2 constitute the major hyaluronidases in somatic tissues and are important in degradation of native HA. In mouse, a seventh gene has been discovered and is referred to as HYAL-5, a candidate enzyme involved in sperm penetration through the cumulus [39]. It has been proposed that HA is bound to cell surfaces by the combined effect of HYAL-2 (bound to the membrane by a glycosylphosphatidylinositol link) and CD44 (Figure 1). This complex is guided into caveolae-rich lipid rafts where HA is internalized and cleaved to LMW fragments (~50 dissaccharides). Such fragments are delivered into lysosomes where fragmentation continues through the action of acid-active HYAL-1 and the exoglycosidases [40, 41]. It has been recently described that in liver cells, active HYAL-1 could be originated by endocytosis of the enzyme present in the serum [42]. In humans, deficiency of HYAL-1 leads to HA accumulation and development of mucopolysaccharidosis IX, a very rare disorder that has been described in few patients [43, 44]. In mice, HYAL-1 deficiency is characterized by joint abnormalities with HA accumulation in chondrocytes [45]. While HYAL-2 deficiency has never been observed in humans, HYAL-2-deficient mice have been generated through a conditional Cre-lox system. These mice are viable and fertile, but exhibit localized congenital defects in frontonasal and vertebral bone formation and suffer thrombocytopenia [46]. HYAL-3 is widely expressed but little is known about its function. It is differentially expressed in mammalian testis and bone marrow [38] and recent data suggest that its presence in sperm contribute with PH-20 in cumulus penetration and the induction of the acrosome reaction [47]. HYAL-3$^{-/-}$ mice are viable and present no GAG accumulation, which suggests that under normal circumstances HYAL-3 does not contribute significantly to HA degradation [48]. However, in HYAL-1$^{-/-}$ mice, HYAL-3
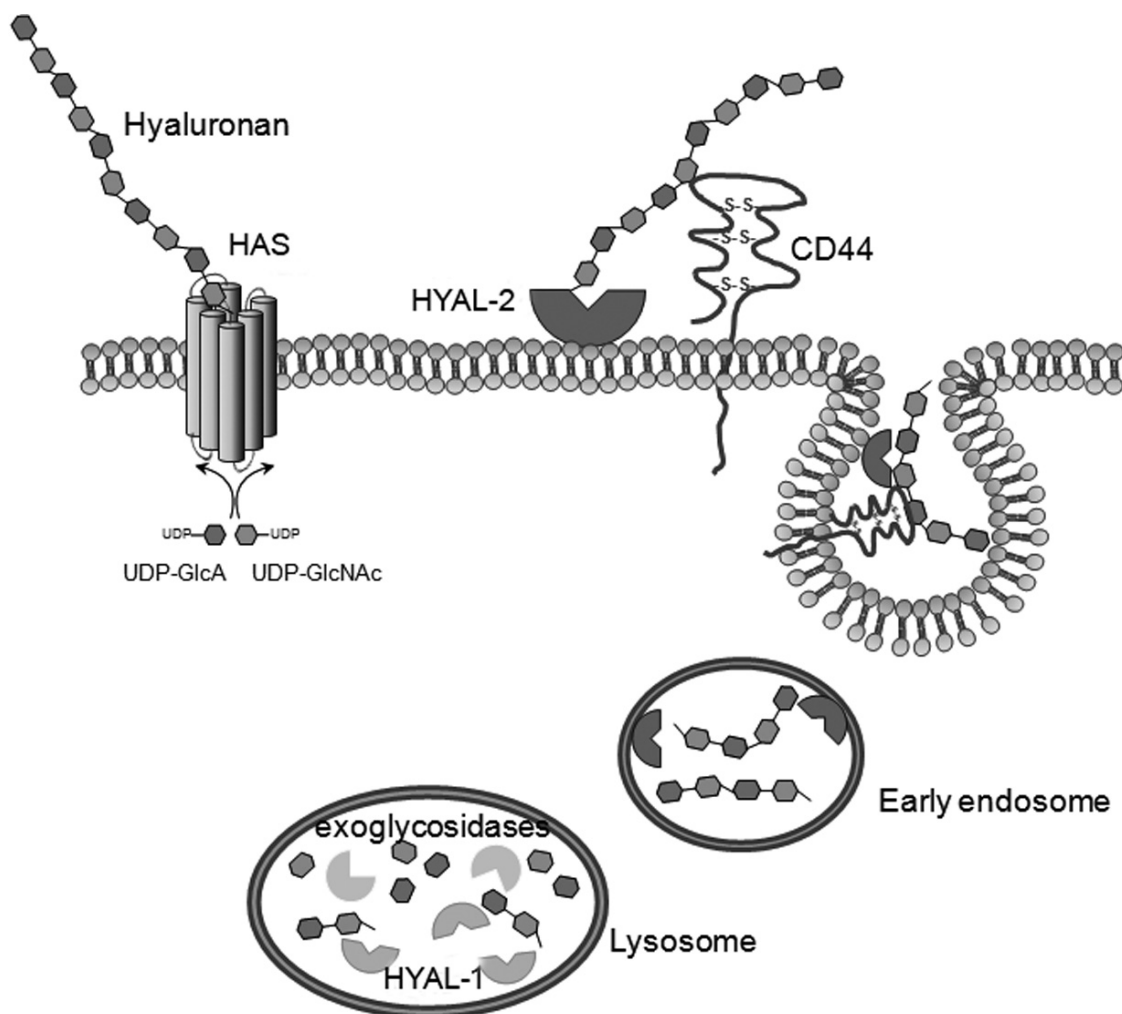
Figure 1. Cellular mechanisms driving HA biosynthesis and degradation. Hyaluronan synthases (HAS) are membrane-bound enzymes which use UDP-α-N-acetyl-D-glucosamine and UDP-α-D-glucuronate as substrates to produce HA, which is then extruded through the membrane into the extracellular space. The three mammalian HAS described to date (HAS-1, -2, -3) differ mainly on the size of the HA molecules they synthetize. In turn, degradation of HA in physiological conditions is mostly driven by the concerted action of the hyaluronidases HYAL-1 and HYAL-2. Membrane bound HYAL-2 captures HMW-HA assisted by the CD44 receptor, forming a complex which is guided to caveolae-rich lipid rafts and internalized. HA fragments resulting from HYAL-1 activity (~50 disaccharides) are further degraded by lysosomal HYAL-1 and exoglycosidases, which remove the terminal sugar residue and generate GlcA and GlcNAc. Normal HA turnover thus results from the balance between HAS and HYAL activities, which are regulated by growth factors, cytokines and other signals derived from the cellular microenvironment.

may compensate HYAL-1 activity in non-skeletal tissues [45], indicating that in pathological conditions HYAL-3 contributes to HA catabolism. Moreover, HYAL-3 expression could stimulate HYAL-1 activity [49]. HYAL-4 is a chondroitinase with no activity against HA and its expression is restricted to placenta and skeletal muscle [38, 50]. PH-20 is found mainly in the sperm and it facilitates penetration and fertilization of the ovum [51].

It is noteworthy that HA can also be degraded by nonenzymatic reactions, caused by free radicals and reactive oxygen species (ROS) [52]. However, the contribution of the enzymatic or nonenzymatic cleavage to HA catabolism in physiological and pathological conditions is not elucidated yet.

HA deposition in tissues is determined by a balance between the expression of enzymes involved in HA synthesis and degradation. Alterations in HAS-HYAL balance leads to altered HA production that is characteristic of pathological conditions such as tumorigenesis and pregnancy-associated pathologies (Figure 2).

How can HA influence tumor progression?

Interactions between tumor cells and their stroma are crucial to tumor growth. Tumor and stromal cells release growth factors and cytokines that increase HA deposition and activate tumor cell expression of their receptors, such as CD44, thus favoring tumor invasion and metastasis [3]. The role of HA in tumor progression is complex and depends mainly on its molecular weight (HMW-HA, LMW-HA or oHA), its interactions with different proteins called hyaladherins and its three-dimensional structure [9]. Upon interaction with surface receptors like CD44, RHAMM, or TLR-4, HA modulates cell proliferation, apoptosis, adhesion, migration, differentiation and multidrug resistance [3, 53]. HA activates receptor tyrosine kinases (such as ErbB-2 and c-Met) and their downstream signaling pathways lead to increased cell survival and proliferation of colon, prostate and breast tumor cells [54]. Interaction of CD44 with HA promotes tumor cell dissemination through promotion of microRNA signaling, activation of Rho GTPases, and the recruitment of the cytoskeletal protein ankyrin to the membrane [55]. Besides, HA is a modulator of the immune system by regulating antigen presentation and dendritic cell (DC) and T cell function [56, 57]. During inflammation, HA undergoes degradation into oligosaccharides (oHA), which are activators of DC and macrophages *in vitro* [2, 58]. Recently, it has been demonstrated that LMW-HA inhibits colorectal carcinoma growth *in vivo* by decreasing tumor cell proliferation and stimulating DC activation and lymphocyte recruitment in the tumor [59]. On the contrary, HMW-HA promotes persistence of induced regulatory T cells [60].
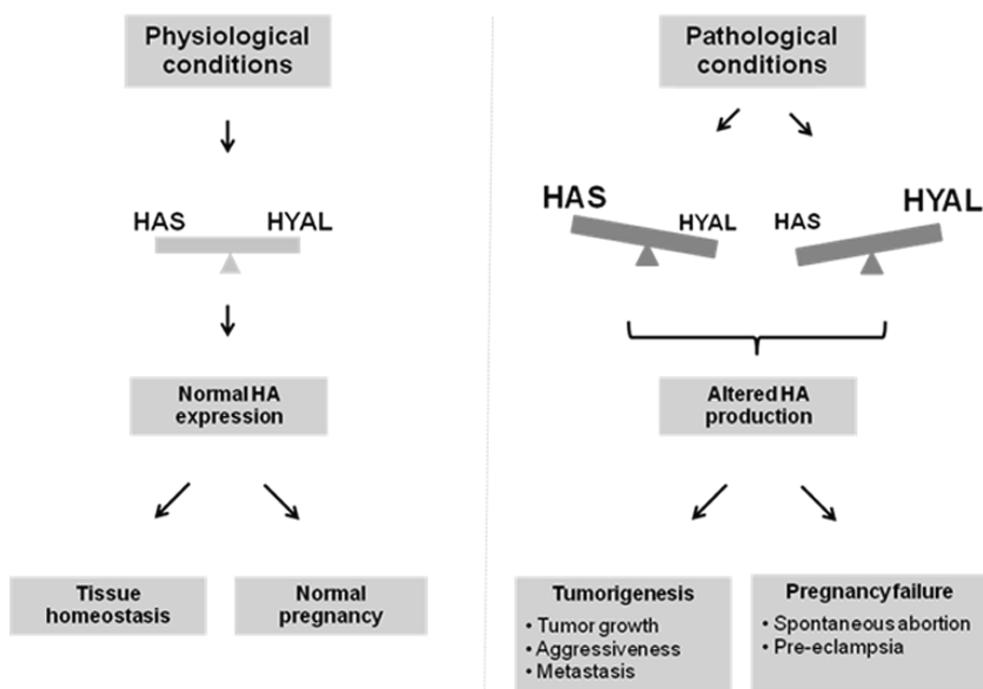

Figure 2. In physiological conditions there is a balance between the expression of enzymes involved in HA synthesis (HAS) and degradation (HYAL) that leads to normal hyaluronan (HA) expression and tissue homeostasis and normal pregnancy. However, alterations in HAS and HYAL balance leads to altered HA production that is characteristic of pathological conditions such as tumorigenesis and pregnancy complications.

However, HMW-HA might exert an antitumoral effect when it is exogenously administered. Injection of HMW-HA inhibited tumor growth in a human breast cancer xenograft model, suggesting that exogenous HA might disrupt endogenous HA binding resulting in tumor regression [61]. Besides, HMW-HA also delayed post-chemotherapy tumor regrowth in colon carcinoma by induction of apoptosis and inhibition of tumor proliferation. Moreover, it has been shown that exogenous HA facilitates the recovery of hematopoiesis after chemotherapy [62]. Despite intensive studies conducted thus far, the complete function of HA molecules is not fully understood. Some of the paradoxical effects of HA could be related to the source of the HA used in the experiments, such as recombinant HA, human umbilical cord HA or rooster comb HA. Besides, when exogenous oHA are used in *in vitro* studies, the method of production and the molecular weight of oHA are not always informed, thus compromising interpretation of the results. As well, if oHA preparations comprise fragments of the same average size, they may have a different range of sizes. Since nowadays oHA of defined numbers of oligomers are commercialized, we believe that ongoing and future research will unreveal past discrepancies. All of this clearly denotes the complexity of HA signaling and the necessity of *in vivo* validation of *in vitro* experiments. Some works have already considered this issue and studied the quality of HA molecules *in vivo*. Indeed, it has been shown that HA fragments isolated from the serum of patients with acute lung injury (approximately 200 kDa LMW-HA) stimulated macrophages to produce inflammatory mediators, thus indicating that HA degradation products generated *in vivo* would induce similar effects to those displayed by exogenous administration *in vitro* [63]. However, further research correlating *in vitro* and *in vivo* findings are required. It should be very interesting to directly identify the molecular weight of HA in the tumor microenvironment, the proportion of each form and the correlation with its biological function.

Altered HA turnover in cancer

Several data demonstrated that HA metabolism is altered in cancer. HA levels are increased in different tumors such as breast, ovarian, prostate, pancreatic, colon and lung cancer as well as in hematological malignancies [64]. HA overexpression in the tumor microenvironment promotes tumor progression and metastasis [65] and increased serum HA levels appear to correlate with tumor metastasis [66-68]. Moreover, serum HA measurement would be a useful cancer prognostic marker in gastrointestinal tumors, oral carcinoma and myeloma [69-71].

Differential expression of HAS and HYAL is associated with increased HA. HAS overexpression, that results in enhanced HA levels, stimulates tumor progression in melanoma, multiple myeloma, colon, breast, ovarian and prostate tumor cells [72-77] and has been positively correlated with tumor aggressiveness. Indeed, aggressive breast and ovarian cancer cells express higher HAS-2 and HAS-3 levels than non-aggressive ones [30, 78], whereas metastatic prostate and colon tumor cells express higher levels of HAS-3 than of HAS-2 [79, 80]. Elevated HAS-1 expression correlates with poor prognosis in human colon, ovarian, endometrial, and bladder cancer, and in multiple myelomas [72, 74-76, 81] and up-regulation of HAS-1 and HAS-2 has been also reported in breast cancer cells [82] and melanoma [77]. Besides, overexpression of HAS-2 and HAS-3 results in excess of HA and enhanced tumorigenicity of mesothelioma and prostate cancer cells respectively [73, 83]. It was recently demonstrated that breast cancer stem cells overexpressed HAS-2, thus generating a favorable microenvironment to bone metastasis [84]. Furthermore, suppression of HAS-2 decreases HA production and reduces tumorigenicity of prostate and breast cancer cells [85, 86]. Moreover, inhibition of HAS-3 decreases colon cancer growth *in vivo* [87, 88].

Recently, it has been observed that 4-Methylumbelliferone (4-MU), an inhibitor of HA synthesis, displays an anti-tumoral effect on melanoma, breast, ovarian and squamous carcinoma cells by depletion of cellular UDP-GlcA and down regulation of HAS-2 and HAS-3 [89]. *In vivo* administration of 4-MU inhibited the expansion of osteolytic lesions in a mouse breast cancer xenograft model, suggesting its potential as therapeutic candidate for bone metastasis of breast cancer [90]. In prostate cancer cells, 4-MU also

exerted an anti-tumoral effect by induction of apoptosis and inhibition of cell growth [91]. Similar results were observed in a murine model of hepatocellular carcinoma associated to fibrosis: 4-MU treatment was able to reduce liver micrometastasis and fibrosis [92]. Moreover, it was recently shown that of 4-MU inhibited osteosarcoma tumorigenesis and lung metastasis *in vivo* [93] as well as reduced the metastasic lesions of breast cancer stem cells [84].

On the other hand, degradation of HA through increased HYAL expression generates HA fragments (oHA), which would be able to inhibit signaling pathways induced by native HA (HMW-HA) and would decrease tumor growth. Indeed, hyaluronidase treatment in animals bearing-breast tumors reduce both HA levels and tumor mass [94], while administration of oHA alone or in combination with chemotherapy agents reduces tumor cell proliferation, induces apoptosis, inhibits tumor growth *in vivo* and reduces chemotherapy resistance by different mechanisms such as inhibition of survival signaling (PI3K/Akt) and modulation of multidrug transporter activity [95-98]. HYAL-1 overexpression suppresses tumorigenicity of colon carcinoma cells [99]. Similarly, low activity of hyaluronidases is associated with the aggressiveness of ovarian epithelial tumors [66] and HYAL-2 underexpression was correlated with higher-grade lymphoma [100]. In some tumors, however, overexpression of HYAL has been associated to negative prognosis. Indeed, HYAL-1 is increased in bladder and prostate tumors [101, 102], as well as in head and neck squamous carcinoma cells [103]. Evidences indicate that HYAL-1 is involved in tumor growth and angiogenesis [104, 105]. Besides, HYAL-2 is overexpressed in breast cancer and is associated with increased aggressiveness and metastasis [106]. Although HYAL-3 expression has been found in endometrial, breast, bladder and prostate tumor cells, its role has not been assessed yet [106-108]. HYAL-3 was also found in advanced stages of colorectal cancer, where it seems to have a synergistic action with HYAL-1 and PH-20 [109]. Recently, it has been demonstrated that HYAL-2 and HYAL-3 mRNA are decreased in higher metastatic lymphoma cells explaining their higher HA production [110]. The

role of HYAL in cancer is complex since it could act as either tumor promoter or suppressor depending on its concentration, location and the tumor cell context. This indicates that further studies are required to completely clarify the role played by HAS and HYAL in tumor progression.

Although several studies on hyaluronan biosynthesis and cancer progression were performed, some questions remain unsolved as to the quantity of HA fragments generated *in vivo* and their influence over native HA action in tumor progression. Despite HA fragments have been detected in injured tissues or in pathological conditions, native HA is also present, and the prevalence of one of them over the other is uncertain. Moreover, we have recently observed in liver cancer associated to fibrosis high HA accumulation without finding clear differences in its molecular weight [92]. Especially in cancer, a high prevalence of HA fragments could be beneficial due to its immunostimulatory capacity, enhanced chemosensitivity and inhibitory growth action on different tumor cells. However, it is known that the tumor microenvironment has immunosuppressive properties and the action of HA fragments in this context is unknown. Thus, studying HA biology in other processes such as normal pregnancy, which share molecular features with cancer, will allow us to clarify some of these questions.

HA metabolism in pregnancy: Where and what for?

Differences in HA deposition and distribution have been observed during murine normal pregnancy. HA increases in response to hormones during implantation favoring invasion of the endometrial stroma [111]. Thus, adequate HA levels at gestation day 4 facilitate implantation because of its ability to expand the ECM and allow blastocyst adhesion [17]. Interestingly, after implantation there is a clearance of HA in the anti-mesometrial decidua, which would be part of a maternal program to restrict trophoblast invasion [17, 18].

Serum HA levels increase during pregnancy especially in the third trimester and at delivery [112, 113] (Figure 3). Besides, HA synthesis is increased in the uterine cervix at late pregnancy,
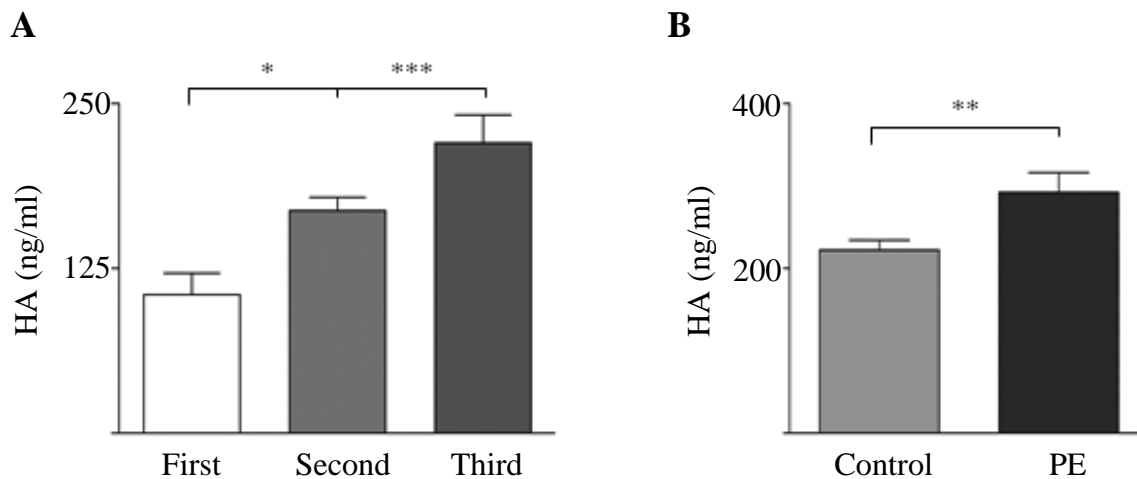
A   **B**

Figure 3. Mean serum HA concentrations in normal and pathological pregnancies, as measured by ELISA. (A) HA levels increase throughout normal human pregnancy, reaching maximal levels during the third trimester. (B) Serum HA concentrations are significantly increased in preeclamptic (PE) women compared to normotensive pregnant controls. Statistical significance is denoted as * *p<0.05*, ** *p<0.01* and *** *p<0.001*, according to the Mann-Whitney U test.

which is probably related to cervical ripening necessary for delivery [114]. Thus, HA is implicated in two important features of cervical ripening: (a) increased tissue hydration and flexibility of the cervix, and (b) increased cervical inflammation by recruitment or activation of inflammatory cells [115]. HAS-1 and HAS-2 are increased in mouse cervix at delivery and this is related to serum HA increase at late pregnancy [112]. Besides, increase in cervical HA correlates with increase of HAS-2 both in mice and humans [116, 117]. In fact, mice deficient in the steroid 5-alpha-reductase type 1 gene, which fail to undergo cervical remodeling, exhibit decreased HAS-2 expression and decreased tissue HA, showing that regulation of HA biosynthesis during cervical ripening is essential to this process [117]. In the mouse cervix, HA is predominantly HMW-HA before birth and LMW-HA after birth [114]. Indeed, HYAL-1 and HYAL-2 increase prior to or during labor. This was suggested to disrupt HA cervical tensile strength and integrity required for cervical dilation and birth. However, LMW-HA might influence activation of the pro-inflammatory process during postpartum tissue repair [114]. Besides, treatment with HYAL reduces the length of labor and the frequency of cesarean delivery in women [118]. To summarize, HA appears to modulate matrix structure and

perhaps inflammatory processes during cervical ripening and postpartum repair.

HA and pregnancy complications

Despite being critical to the survival of the species, mammalian reproduction is relatively inefficient. Only about 40% of pregnancy losses is attributable to known causes such as chromosomic anomalies, endocrinological abnormalities, or anatomical problems, but the other 60% are of unknown etiology [119]. Spontaneous abortion (SA) is one of the most common complications of pregnancy, and the abortion prone CBA/J x DBA/2J mouse model provides a valuable tool for defining mechanisms leading to pregnancy loss [120]. Immunization of CBA/J females with lymphoid cells of BALB/c males reduces abortion rates [121], thus indicating the presence of an immunological component in this pathology. Moreover, it has been observed that secretion of the Th1- cytokines IFN-gamma and TNF-alpha plus a TLR signal would increase levels of prothrombinase fg12, which converts prothrombin in thrombin. Thrombin can stimulate neutrophil recruitment, contributing to embryonic demise [122]. Besides, increased Th1-cytokine levels produced by macrophages, NK and T cells induce an inflammatory process with deleterious effects on pregnancy, while an anti-inflammatory response

induced by Th2 cytokines would be pregnancy-protective [123]. Interestingly, we found increased decidual HA levels in SA (DBA/2J-mated CBA/J females) compared with normal pregnancy (BALB/c-mated CBA/J females) on gestation day 7.5 (peri-implantation period), which is a critical time period for fetal survival in this mouse model. Both in normal and pathologic pregnancies, HMW-HA was found at the feto-maternal unit [124]. However, HA metabolism was altered in SA as shown by a decrease in HYAL-3 expression and by differences in the molecular size distribution of HA. This alteration in HA metabolism could explain the increase of HA levels and its abnormal distribution around the embryo implantation crypt. Thus, increased decidual HA levels resulting from abnormal deposition and turnover may contribute to the pathogenesis of SA [124].

Spontaneous abortion has been related with immunological rejection of the fetus and HA modulates the immune system. HA synthesis is induced by inflammatory stimulus such as LPS, IL-1beta, TNF-alpha and IL-15 [56]. Besides, oHA -as well as LMW-HA-induce DC maturation through TLR-4 and/or CD44 receptors [58, 125, 126]. In addition, HMW-HA stimulates the secretion of TNF-alpha, IL-1beta and IL-8 by uterine fibroblasts [127], showing that HMW-HA has differential effects on different models. Moreover, in normal pregnancy low levels of HA in decidua alter DC migration towards lymph nodes, minimizing exposure of fetal antigens and favoring immunological tolerance [128]. In this context, enhanced HA levels around the embryo crypt in SA [124] would generate alterations in ECM-cell interactions leading to increased exposure of fetal antigens, activation of the immune system and contributing to pregnancy failure. Whether HA accumulation is the cause or the consequence of pro-inflammatory signals leading to pregnancy failure, and the mechanisms by which HA contributes to abortion remain to be elucidated. Angiogenesis is an important process in pregnancy and it has been discovered that DC induce angiogenesis, thus favoring implantation, decidualization and placentation [129]. However, DC may also inhibit angiogenesis through the release of ECM components (such as thrombospondin-1 and pentraxin-3) that interfere

with angiogenic factors [130]. Besides, the angiogenic activity of HA depends on its molecular weight: oHA have been implicated in the promotion of angiogenesis [88, 131], while HMW-HA has been described as an anti-angiogenic factor because of its ability to inhibit endothelial cell proliferation and migration and capillary formation in three-dimensional matrixes [88, 132]. Since DC synthesize HA [56], we hypothesize that excess of HMW-HA surrounding the embryo crypt in SA would also function as an anti-angiogenic factor with deleterious effects on placental blood supply leading to miscarriage. Further experiments are required to dissect the contribution of HA in angiogenesis associated with gestation.

Preeclampsia is another complication of pregnancy that causes maternal and neonatal mortality and that manifests with increased blood pressure and proteinuria after 20 weeks of gestation. Recently, pathogenesis of preeclampsia has been related to inflammation [133] and even the CBA/J x DBA/2J murine model of SA has been shown to mimic the symptoms of preeclampsia patients such as elevated blood pressure and proteinuria [134]. Serum HA levels are increased in women with preeclampsia [135, 136] (Figure 3), and there is indeed evidence that such increase would result from altered placental expression associated to the formation of perivillous fibrinoid deposits [136, 137]. In this context, investigation of HYAL and HAS activity in preeclampsia patients would greatly improve the understanding of the contribution of HA metabolic pathways to this complex syndrome.

CONCLUSIONS AND PERSPECTIVES

Several reports indicate that HA has an important role both in pathological and physiological processes such as in tumorigenesis and pregnancy. Since the synthesis of HA stimulates tumor growth and metastasis, suppression of its synthesis and/or blockage of HA binding to its receptors constitutes an interesting therapeutic strategy to cancer treatment. Indeed, it has been demonstrated that treatment with anti-CD44 antibodies, oHA or inhibition of HA synthesis with 4-MU induces apoptosis, tumor regression, and modulation of the immune system.

The balance between HA synthesis and degradation also plays a complex role in tumors and pregnancy outcome. In cancer, there are several studies linking HAS or HYAL activity with tumor progression but in some cases the results are paradoxical. Thus, a complete understanding of the involvement of HAS and HYAL in cancer *in vivo* still requires substantially more research, especially in clinical application. In SA, more studies are needed to unravel the mechanisms by which HA contributes to abortion. Special concern should be taken in relation to the ability of HA to modulate the immune system. As we have explained above, HA of different molecular weight have differential effects on cells of the immune system (e.g. DC or macrophages) and even polymers of the same molecular weight exhibit different functions on different models [8, 58, 125, 127]. Further studies are required to completely elucidate the mechanisms by which HA modulates the immune response both in tumors and pregnancy pathologies.

Since alterations of the balance of HAS and HYAL have been observed both in tumors and pregnancy-associated pathologies, restoring the normal balance of HAS and HYAL - for example, by gene therapy techniques - could be an interesting strategy for cancer treatment or prevention of pregnancy losses.

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CONFLICT OF INTEREST

None declared.

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