Targeting hyaluronan for the treatment of pancreatic ductal adenocarcinoma

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Abstract Progression of cancer is often associated with interactions between cancer cells and extracellular matrix (ECM) surrounding them. Increasing evidence has suggested that accumulation of hyaluronan (HA), a major component of ECM, provides a favorable microenvironment for cancer progression. Pancreatic ductal adenocarcinoma (PDAC) is characterized typically by a dense desmoplastic stroma with a large amount of HA, making this molecule as an attractive target for therapy. Several studies have shown efficacy of inhibitors of HA synthesis or signaling for the treatment of PDAC. Recent studies have also demonstrated substantial improvements in the effects of chemotherapy by a targeted depletion of stromal HA in PDAC using an enzymatic agent. Thus, targeting HA has been recognized as a promising therapeutic strategy to treat this highly aggressive neoplasm. In this review article, we summarize our current understanding of the role of HA in the progression of PDAC and discuss possible therapeutic approaches targeting HA.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and intractable solid tumors, which often invades surrounding stromal components, including lymphatic, vascular, and perineural systems, ultimately metastasizing to distant organs. Despite recent advances in the clinical management, the survival rate in patients with PDAC remains the lowest among all cancer types, emphasizing the need for a better understanding of its biology. In particular, identification of molecular mechanisms underlying the aggressive behaviors of PDAC can provide the basis for the development of targets for therapeutic intervention. Although substantial progress has been made in our understanding of the genetic and epigenetic alterations in PDAC, the identification of these molecular defects in cancer cells has led to little progress in developing new treatment strategies.

The progression of cancer is governed by complex mechanisms and is significantly accelerated by tumor microenvironment composed of extracellular matrix (ECM), such as collagen, fibronectin, laminin, and hyaluronan (HA). Among the ECM components, HA has been extensively studied in its relation to cancer initiation and progression. HA, a large polysaccharide composed of repeating disaccharides of glucuronic acid and N-acetyl-glucosamine, plays a critical role in a variety of cellular processes. HA regulates cell adhesion, migration, and proliferation by interacting with specific cell surface receptors including CD44 and receptor for HA-mediated motility (RHAMM). HA is synthesized by hyaluronan synthases (HAS, including HAS1, HAS2, and HAS3) and is degraded by hyaluronidases (such as HYAL1 and HYAL2). In normal physiological conditions, the amount of HA is controlled by a balance between synthesis and degradation. In certain cancers, HA is often increased or highly concentrated in tumor cells and, particularly, in their surrounding ECM. There have been several studies investigating the degree of HA concentration and/or pattern of HA expression in PDAC. For example, a previous study showed that HA is secreted from cultured human pancreatic cancer cell lines. In addition, the amount of HA is increased in human PDAC tissues (12-fold increase) as compared to the normal pancreas. Using a biotinylated HA-binding protein isolated from bovine cartilage, Fries et al. demonstrated that in primary PDAC tissues, HA was found predominantly in the connective tissue immediately around tumor cells or at the border between the tumor and normal pancreatic tissue. A comprehensive analysis of the HA content in a variety of human malignant tumors revealed that PDAC had the highest incidence of detectable HA content, which was predominantly associated with the desmoplastic stroma rather than with tumor cells. We also used immunohistochemistry to analyze the expression of HA and its regulators (including HAS2 and HYAL1) in primary PDAC, and demonstrated that HA is strongly expressed in 80% of primary PDAC tissues with a staining being detected both in tumor and stromal components. Importantly, strong HA expression was an independent prognostic factor in patients with PDAC undergoing resection, suggesting a prognostic significance of HA in PDAC.

Little is known about the mechanism by which HA is aberrantly accumulated in PDAC. One possible mechanism is increased production of HA from cancer cells themselves through its accelerated synthesis. In fact, our previous study demonstrated overexpression of one of the HA synthases, HAS2, in PDAC tissues. We and other researches also demonstrated that cultured PDAC cells secrete a certain amount of HA into conditioned media. Furthermore, we recently discovered that epigenetic mechanism (namely DNA methylation) is involved in the regulation of HA synthesis in PDAC cells. Another mechanism may be related to the enhanced secretion of HA from stromal cells, including fibroblasts. In support of this, it has been shown that HA staining was predominantly associated with the desmoplastic stroma rather than with tumor cells in human PDAC tissues. Increased in stroma, HA staining is observed mainly in tumor cells (arrows) but is also present in stroma (*).

2. Role of HA in the progression of PDAC

HA is a large, linear glycosaminoglycan that consists of repeating disaccharide subunits of glucuronic acid and N-acetylglucosamine produced by HA synthases (HAS1, HAS2, and HAS3) and degraded by hyaluronidases (mainly HYAL1 and HYAL2). In normal physiological conditions, the amounts of HA in tissues are tightly regulated by a balance between synthesis and degradation. In certain cancers, HA is often increased or highly concentrated in tumor cells and, particularly, in their surrounding ECM. There have been several studies investigating the degree of HA concentration and/or pattern of HA expression in PDAC. For example, a previous study showed that HA is secreted from cultured human pancreatic cancer cell lines. In addition, the amount of HA is increased in human PDAC tissues (12-fold increase) as compared to the normal pancreas. Using a biotinylated HA-binding protein isolated from bovine cartilage, Fries et al. demonstrated that in primary PDAC tissues, HA was found predominantly in the connective tissue immediately around tumor cells or at the border between the tumor and normal pancreatic tissue. A comprehensive analysis of the HA content in a variety of human malignant tumors revealed that PDAC had the highest incidence of detectable HA content, which was predominantly associated with the desmoplastic stroma rather than with tumor cells. We also used immunohistochemistry to analyze the expression of HA and its regulators (including HAS2 and HYAL1) in primary PDAC, and demonstrated that HA is strongly expressed in 80% of primary PDAC tissues with a staining being detected both in tumor and stromal components (Fig. 1). Importantly, strong HA expression was an independent prognostic factor in patients with PDAC undergoing resection, suggesting a prognostic significance of HA in PDAC.

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Figure 1 Overexpression of hyaluronan in human pancreatic ductal adenocarcinoma tissue by immunohistochemical staining. Strong staining is observed mainly in tumor cells (arrows) but is also present in stroma (*).
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3. Therapeutic strategies targeting HA in PDAC

In light of these critical contributions of HA to tumor progression, there have been great interest in developing therapeutic strategies targeting HA. Three different therapeutic approaches may be identified: (1) inhibiting HA synthesis, (2) blocking HA signaling, and (3) depleting stromal HA in PDAC to improve chemosensitivity (Fig. 2).

3.1. Targeting HA synthesis

First, inhibition of HA synthesis may be an ideal and straightforward treatment strategy. One agent that has received increasing attention is 4-methylumbelliferone (4-MU). 4-MU inhibits HA synthesis by acting as a competitive substrate for UDP-glucuronosyltransferase (UGT) and by downregulating HAS2 and HAS328,29. Notably, 4-MU, also known as hymecromone, is already used in several countries as a drug to improve liver fibrosis in human beings30. However, these studies used cancer cell lines and/or xenograft models which basically lack human tumor stroma, raising a concern about the potential therapeutic benefits of 4-MU in patients with PDAC. Further preclinical and clinical studies are required to determine the efficacy of 4-MU for the treatment of PDAC.

3.2. Targeting HA signaling

In addition to the synthesis process of HA, signal transduction pathways induced by HA can be a target for anticancer therapy. HA interacts with several cell-surface receptors, including CD44 and RHAMM, to activate intracellular signaling pathways that regulate a variety of cellular processes. In many cancers including PDAC, HA-CD44 interactions may play an important role in tumor cell growth, survival, migration, invasion, multidrug resistance, and cancer stem cell self-renewal31–33. Previous studies34,35 have shown that CD44 regulates invasion of PDAC. Therefore, blocking this interaction may prevent progression of PDAC. For example, Sugahara et al.36 showed that tumor-derived HA fragments enhance CD44 cleavage and cell migration in a CD44-dependent manner and that inhibition of CD44-HA interaction by digesting the HA oligosaccharides using hyaluronidase results in complete abrogation of these cellular events. As downstream of CD44-HA interactions, Ras-mitogen-activated protein kinase pathway and phosphoinositide 3-kinase (PI3K)-Akt signaling pathway are known to be involved in the cancer-promoting effects of HA37. These signaling pathways are thus a target for therapy. Teranishi et al.38 demonstrated that enhanced cell motility and peritoneal metastasis of PDAC induced by HA were blocked by the PI3K inhibitor wortmannin in mice.

RHAMM is another receptor for HA known to be implicated in various cancers. RHAMM mRNA is overexpressed in pancreatic cancer cell lines exhibiting a poorly differentiated phenotype and a high metastatic potential when injected into nude mice15. Recently, we also show that RHAMM is overexpressed in primary PDAC tissues and its expression correlates with poor survival in patients who underwent surgical resection17. Therefore, RHAMM may also be a promising target but has not yet been investigated in terms of its therapeutic efficacy in PDAC.

3.3. Depleting stromal HA in PDAC

PDAC is characterized typically by its extensive fibrosis in a stromal region as a result of desmoplastic reaction. It has been suggested that accumulation of HA in tumor stroma may increase tumor interstitial pressure, thereby blocking delivery of drugs to the tumor cells. Consequently, targeting the components of ECM, particularly HA, has been considered an attractive therapeutic strategy to overcome chemoresistance15,42. Although this idea of depleting stromal HA has been previously proposed and tested in other tumor types43, it had not been tested in a model of PDAC until recently. Provenzano et al.18 investigated intravenous administration of PEGPH20, an HA-targeting enzymatic agent, in mice bearing PDAC. Systemic administration of PEGPH20 depleted stromal HA, normalized interstitial pressure, re-expanded microvasculature, and consequently improved the effects of gemcitabine46. Similarly, Jacobetz et al.21 also used a genetically engineered mouse model, the LSL-Kras<sup>G12D/+</sup>; LSL-Trp53<sup>R172H/+</sup>; Pdx-1-Cre (KPC) mice, to demonstrate that PEGPH20 depletes HA, induces the re-expansion of collapsed blood vessels in PDA, and increases the intratumoral delivery of two chemotherapeutic agents, doxorubicin and gemcitabine. Furthermore, combination therapy with PEGPH20 and gemcitabine inhibits tumor growth and prolongs survival in the metastasis-prone KPC background47.
KPC mice. Importantly, treatment with PEGPH20 alone had no significant effects on the tumor growth and survival in mice, suggesting that the potential therapeutic benefit of HA inhibition is obtained primarily by overcoming the stromal barrier and sensitizing chemotherapy rather than by its own anticancer effect.

Based on these promising results of preclinical studies, PEGPH20 is now being tested in a clinical trial (NCT01839487) to determine its efficacy when used in combination with nab-paclitaxel plus gemcitabine in patients with metastatic PDAC (https://clinicaltrials.gov/show/NCT01839487). The results of this and future trials will reveal the clinical efficacy of HA inhibitors and offer a novel treatment option for otherwise untreatable patients with PDAC.

4. Future prospective

In summary, there are currently three major strategies targeting HA (inhibition of HA synthesis, blocking HA-receptor signaling, and depletion of stromal HA in combination with chemotherapy) in the treatment of PDAC. In addition to these strategies, there may be other potential strategies to target HA for the treatment of PDAC. For example, inhibition of HA degradation, as well as HA synthesis, could be an ideal strategy, because accumulating evidence suggests that low-molecular-weight or fragmented HA, produced through degradation by hyaluronidase, plays a critical role in cancer progression. In fact, previous studies have shown antitumor effects of hyalurondase inhibitors in some types of cancers. Although further preclinical and clinical studies are required, controlling the amount and/or size of HA by modulating the production and degradation process may be a promising therapeutic strategy to improve the prognosis of this deadly disease in the future.

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