Hyaluronic acid-based nanocarriers for intracellular targeting: Interfacial interactions with proteins in cancer

Ki Young Choi [a,b,1], Gurusamy Saravanakumar [c,1], Jae Hyung Park [c], Kinam Park [a,*]

[a] Purdue University, Departments of Biomedical Engineering and Pharmaceutics, West Lafayette, IN 47907, USA
[b] Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD 20892, USA
[c] Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea

ARTICLE INFO

ABSTRACT

The therapeutic efficacy of most drugs is greatly depends on their ability to cross the cellular barrier and reach their intracellular target sites. To transport the drugs effectively through the cellular membrane and to deliver them into the intracellular environment, several interesting smart carrier systems based on both synthetic or natural polymers have been designed and developed. In recent years, hyaluronic acid (HA) has emerged as a promising candidate for intracellular delivery of various therapeutic and imaging agents because of its innate ability to recognize specific cellular receptors that overexpressed on diseased cells. The aim of this review is to highlight the significance of HA in cancer, and to explore the recent advances of HA-based drug carriers towards cancer imaging and therapeutics.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Significant research efforts have been devoted over the past few decades to design carrier systems that could specifically deliver active agents to the disease sites, and thereby minimizing the lethal side-effects [1–3]. Recent exciting advances in nanotechnology and our understanding in molecular biology have enabled us to develop a variety of efficient nanocarriers to deliver diagnostic and/or therapeutic agents to the tumor tissue [4–11]. In particular, polymeric nanoparticles have been extensively used for targeted cancer diagnosis and therapy [8,12]. Macromolecules and nanoparticles have been found to passively accumulate into tumor sites after systemic administration due to their abnormally leaky vasculature and lack of an effective lymphatic drainage system, and this phenomenon is referred to as the enhanced permeation and retention (EPR) effect [13]. However, a number of nanoparticles have not been able to show desirable therapeutic efficacy *in vivo* because the EPR effect cannot guarantee internalization of the nanoparticles. Even, considerable portion of drugs may be released from the nanoparticles before they are taken up by the tumor cells. Since the therapeutic targets of many anticancer drugs are found inside the cells, effective cancer therapy requires development of nanoparticles that can accumulate in a tumor tissue, penetrate into cancer cells, and release the drugs inside the cells. Intracellular delivery of anticancer drugs is important for enhanced therapeutic effect.

Intracellular delivery has been improved by conjugating tumor-interacting moieties, such as antibodies [14–16], nucleic acids [17,18], proteins [19–22], and various other ligands [23–25], onto the surface of the nanoparticles. Because such nanoparticles can recognize, bind to, and internalize into tumor cells through endocytosis, diagnostic or therapeutic agents loaded within the targetable nanoparticles can be release inside of the tumor cells [10,11,26,27]. However, many of the tumor-targeting moieties are associated with various complications. For example, the use of antibodies is limited by its immunogenicity, and decrease in the activity due to chemical conjugation processes.

In recent years, hyaluronic acid (HA) has attracted much attention in tumor-targeted delivery because of its ability to specifically bind to various cancer cells that overexpress CD44 receptor [28]. Moreover, HA also possess numerous desirable physicochemical and biological properties such as biocompatibility, biodegradability and non-immunogenicity, for drug delivery applications. Already, a number of drug delivery systems such as drug-conjugates, nanocomplexes, and nanoparticles, using HA as the primary (targeting) constituent have widely investigated. This review consists of three parts: introduce of physicochemical and biological characteristics of HA, including the synthesis, physiological functions,

* Corresponding author at: Purdue University, Weldon School of Biomedical Engineering, 206 S. Martin Jischke Drive West Lafayette, IN 47907-2032, USA.
Tel.: +1 765 494 7759.
E-mail address: kpark@purdue.edu (K. Park).
[1] These authors contributed equally to this work.

cellular interactions, and degradation in the human body; examination of significant roles of HA in cancer; and comprehensive discussion of the recent advances of HA-based drug delivery systems.

2. Hyaluronic acid – chemical structure, biosynthesis, cellular interactions, and biodegradation

2.1. Chemical structure of HA

In 1934, HA was first isolated from the vitreous of bovine eyes by Meyer and co-worker [29]. The name "hyaluronic acid" was coined by them as a conjugation of two words, hyaloid (vitreous) and uronic acid. After nearly 20 years of research, Meyer's group determined the precise chemical structure of HA [30]. They found that HA is a linear polysaccharide composed of a repeating disaccharide of N-acetyl-D-glucosamine (GlcNAc) and D-glucuronic acid (GlcA) with $1 \rightarrow 4$ interglycosidic linkages, while the disaccharide repeating units are linked by β $(1 \rightarrow 3)$ linkages (Fig. 1). In normal physiological conditions, the number of repeating disaccharides in a HA molecule ranges from 2000 to 25,000, which corresponds to a molecular mass of 10^6–$10^7 \rightarrow$ Da (as molecular mass of single disaccharide unit is approximately $400 \rightarrow$ Da). As the pK of the carboxyl groups on the GlcA residue is found to be 3–4, the carboxyl groups are predominantly ionized under physiological conditions (pH 7.4) [31]. Thus, HA exists as a polyanion *in vivo*, and therefore is referred to as hyaluronan. In addition, HA shows an expanded random coil structure in physiological solution. Because of its random-coil structure and high molecular weight (HMW), HA forms very viscose and elastic solution with a large hydrodynamic volume.

2.2. Synthesis of HA by HA synthases

HA is synthesized in the inner face of the plasma membrane by enzyme HA synthases (HASs): HAS-1, HAS-2 and HAS-3, which are multipass transmembrane proteins that composed of hydrophobic amino acid clusters and large cytoplasmic loops. These transmembrane enzymes sequentially link GlcA and GlcNAc using their activated nucleotide sugars, UDP-GlcA and UDP-GlcNAc substrates, in alternating β-1,3 and β-1,4 linkages [28,32,33]. During this synthetic process, HA is secreted outside of the cells onto the cell surface, or into the extracellular matrix (ECM) [28,34]. In addition to cell surface and ECM, HA is also found inside the cell. The pericellular HA, which is anchored to the cell surface by the interaction with the HASs or HA receptors, allows the incorporation of extracellular hyaladherins such as aggrecan into the cell surface.

2.3. Hyaluronic acid-binding proteins: hyaladherins

Besides being an important structural component of tissues in all vertebrates, HA is also implicated in several biological functions including intracellular signaling. The unique biological functions of HA is largely attributed to the specific binding and interaction with HA-binding proteins referred to as hyaladherins [35]. Most

hyaladherins contain a specific binding domain, also called a link module, which is composed of two α-helices and two antiparallel β-sheets [36]. The HA-binding proteins containing the link modules include diverse proteoglycans, HA receptors and a link protein. The proteoglycan molecules can bind to the HA polymer, to form huge proteoglycan complexes, which can acts as a structural components of diverse tissues such as articular cartilage, blood vessels, skin and brain [37–39]. The best-identified HA receptors, containing the link module, are CD44 and lymphatic vessel endothelial HA receptor (LYVE-1). CD44 is known to have a variety of significant biological roles including maintaining tissue structure via cell–cell and cell–matrix adhesion. It also mediates cell migration during morphogenesis, angiogenesis, and tumor invasion and metastasis. CD44 not only organizes matrix signaling related to cell survival and death, but also mediates the adhesion and rolling of lymphocytes [40–46]. The other HA receptor LYVE-1, expressed on the lymph vessel endothelium, is known to participate mainly in HA degradation [47–49]. In addition to the above hyaladherins, tumor necrosis factor-stimulated gene-6 (TSG-6) has also been well identified as a HA-binding protein. TSG-6 is abundantly found in the synovial fluids of arthritis patients and also detected in the serum of patients with inflammatory or autoimmune diseases. It is also known to be involved in inflammation, leukocyte migration and ECM remodeling [50]. In addition, hyaladherins that do not contain the link module have been also identified. The representative examples include inter-α-inhibitor (IαI), CD38, and receptor for HA-mediated motility (RHAMM) that is also known as CD168. Unlike the other hyaladherins, RHAMM is present in the cytoplasm and nucleus and transiently expressed on the surface of activated leukocytes and fibroblasts. RHAMM is known to mediate cell migration and proliferation [45,51–53].

2.4. Interactions between HA and CD44

CD44 is a principal cell-surface receptor for HA, which is widely responsible for the interaction between HA and the surface of specific cells. The interaction between the HA and CD44 has been extensively investigated because of its involvement in a wide variety of cellular functions. In particular, CD44 is closely involved in a variety of significant cellular events, including cell proliferation, cell differentiation, cell migration, angiogenesis, and arrangement of cytokines, chemokines and growth factors to the corresponding receptors. The interaction between HA and CD44 is known to mediate signaling for cell survival and endocytosis of HA for its degradation [40,54,55].

2.4.1. HA–CD44 in cell–cell aggregation
In early 1980, it was recognized that the interaction between HA and a membrane receptor was known to mediate cell–cell aggregation via cross-bridging among pericellular HA matrices and the receptors on the surface of other cells. But the subsequent studies discovered that an integral membrane glycoprotein of 85 kDa was responsible for the HA-cell binding and cell–cell aggregation [56,57]. Later, the membrane glycoprotein, referred as gp85 [58], was finally defined to be identical to a leukocyte homing receptor antigen CD44 [59].

2.4.2. HA–CD44 in cell–matrix signaling
The transmembrane CD44 regulates signaling between cell and the matrix HA. The interaction between cell and the matrix activates intracellular signal via the transmembrane receptor CD44. Conversely, the intracellular signals also regulate changes in the ECM. In some cells, binding of the HA molecule to multiple CD44 receptors activate the receptors and induce the extracellular clustering of the receptors. These extracellular clustering of CD44 regulates intracellular organization of cytoskeleton, resulting in
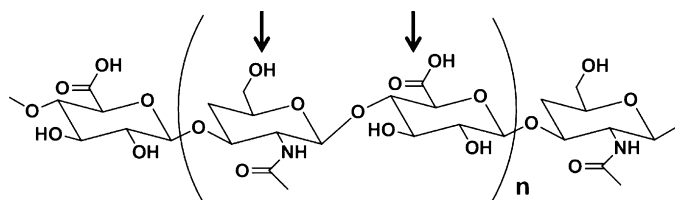

Fig. 1. Chemical structure of HA. The arrows represent principal sites for chemical modification.