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Hyaluronic acid nanoparticles for active targeting atherosclerosis

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ABSTRACT

For the effective diagnosis and therapy of atherosclerosis, there is a pressing need to develop the carrier which can specifically deliver the agents to the pathological site. Since the representative hallmark of atherosclerosis in its pathogenic process is the over-expression of the receptors for hyaluronic acid (HA) such as stabilin-2 and CD44, we herein investigated the potential of HA nanoparticles (HA-NPs) as the carrier for active targeting atherosclerosis. From *in vitro* cellular uptake tests, it was revealed that HA-NPs were selectively taken up by the cells over-expressing stabilin-2 or CD44. On the other hand, the cellular uptake of HA-NPs was drastically reduced when the cells were pre-treated with excess amount of free HA, implying that HA-NPs were taken up by the receptor-mediated endocytosis. Following systemic administration of Cy5.5-labeled NPs into the ApoE-deficient mice as the animal model, the atherosclerotic legion was assessed at 24 post-injection by using the optical imaging system. Interestingly, the fluorescent signal of the atherosclerotic lesion by HA-NPs was much stronger than that of the normal aorta. Three dimensional z-stack images of an atherosclerotic plaque indicated the even distribution of HA-NPs in the atherosclerotic legion. It was demonstrated by immunohistochemistry that HA-NPs were co-localized with the HA receptors including stabilin-2 and CD44. In addition, the amount of HA-NPs, accumulated in the atherosclerotic lesion, was much higher than that of HGC-NPs, known to reach the atherosclerotic lesion by the passive targeting mechanism. Overall, it was evident that HA-NPs could effectively reach the atherosclerotic lesion via the active targeting mechanism after systemic administration, implying their high potential as the carrier for diagnosis and therapy of atherosclerosis.

1. Introduction

Atherosclerosis, characterized by the build-up of lipid-rich plaques within the artery wall, is one of leading causes of cardiovascular disease such as myocardial infarction, stroke, and chronic angina [1–3]. The size and composition of the atherosclerotic plaque are responsible for its pathophysiological behavior [4,5]. In general, vulnerable plaques susceptible to rupture, which are associated with acute syndromes and death, contain a large number of inflammatory cells, large lipid cores, and thin fibrous caps [1,6]. The currently available drugs for atherosclerosis have the functions to alleviate hypertension and hyperlipidemia or to control hemostasis for prevention of thrombotic complications. However, they do not directly suppress the inflammatory mechanisms driving progression of atherosclerosis. Although there are emerging therapeutics such as high-density lipoprotein mimetics and IL-1 receptor antagonist [7–9], their targeted delivery to the atherosclerotic lesion has remained a challenge.

The conventional imaging techniques used to detect atherosclerosis include quantitative coronary angiography, computed tomography, intravascular ultrasound, and magnetic resonance imaging [2]. These techniques are useful to measure the luminal diameter, abnormal narrowing of blood vessels, the thickness of the

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artery wall, and the plaque volume. However, they are often too invasive and do not impart information on functional changes in arterial walls. Therefore, there is an urgent need to develop the improved techniques by using the imaging probe which can specifically accumulate in the atherosclerotic lesion.

Over the last two decades, significant efforts have been made to develop nanoparticles for diagnosis and therapy of atherosclerosis [10]. For high diagnostic and therapeutic efficacy, they should have the ability to identify the targets at the atherosclerotic lesion such as integrins, adhesion molecules, and receptors [2,4]. Therefore, the surfaces of nanoparticles have often been decorated with the targeting moieties including antibodies, proteins, peptide, or other ligands [10–15]. Recently, our group has demonstrated that stabilin-2 (also called HARE), a receptor for hyaluronic acid (HA), is highly expressed in atherosclerotic plaques than in normal vessels [12]. In particular, stabilin-2 was abundant on macrophages, smooth muscle cells, and endothelial cells of atherosclerotic plaques. When polymeric nanoparticles bearing stabilin-2-specific peptide ligand were systemically administered into the low-density lipoprotein receptor-deficient ($Ldlr^{-/-}$) mice, they were selectively accumulated into the atherosclerotic lesion. These results imply that stabilin-2 can be a primary target of atherosclerosis to make the nanoparticles for imaging and therapy.

Owing to its high biocompatibility and biodegradability, hyaluronic acid (HA) has attracted much attention for biomedical applications such as tissue engineering and drug delivery systems [16–18]. In particular, HA-based nanoparticles or nanoconjugates have shown promising potential as the drug carrier for cancer therapy, since HA can specifically bind to CD44 which is overexpressed on various cancer cells [19–23]. Interestingly, CD44 is

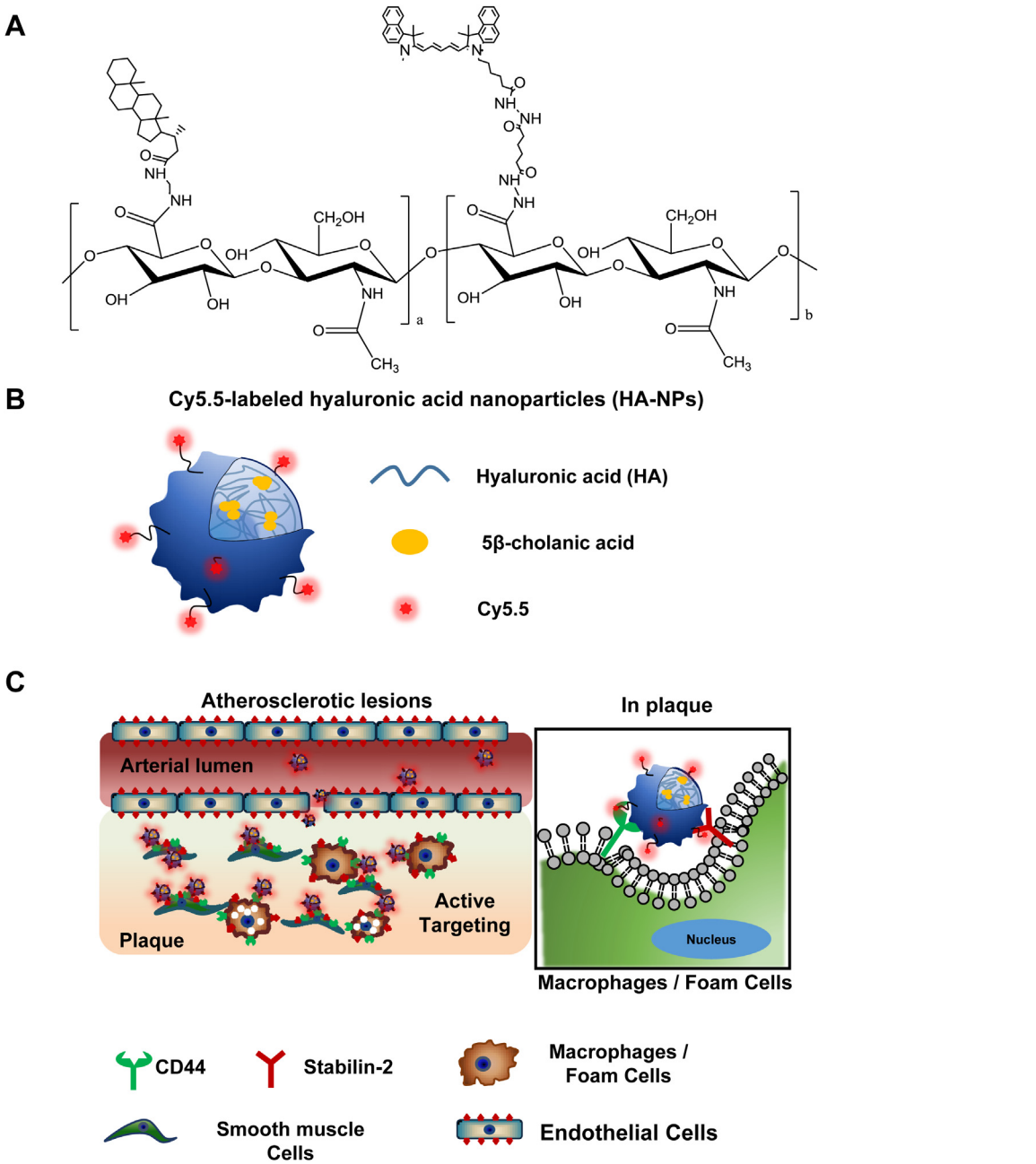

Fig. 1. Development of hyaluronic acid nanoparticle (HA-NPs) for targeting atherosclerosis. (A) Chemical structure and (B) Illustration of HA-NPs. (C) Schematic illustration of active targeting atherosclerosis by HA-NPs.