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Engineering a vascular endothelial growth factor 165-binding heparan sulfate for vascular therapy

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ABSTRACT

The therapeutic use of $VEGF_{165}$ to stimulate blood vessel formation for the treatment of peripheral arterial disease or cardiovascular-related disease has met with limited success. Here we describe an affinity-isolated heparan sulfate glycotherapeutic ($HS7^{+ve}$) that binds to, and enhances the bioactivity of, $VEGF_{165}$. Application of $HS7^{+ve}$ complexed with $VEGF_{165}$ results in enhanced $VEGF_{165}$–VEGFR2 interaction, prolonged downstream pErk1/2 signalling, and increased cell proliferation and tube formation in HUVECs, compared with $VEGF_{165}$ alone. The pro-angiogenic potential of $HS7^{+ve}$ was further assessed *in vivo* using the chick embryo chorioallantoic membrane (CAM) assay. Exogenous dosing with $HS7^{+ve}$ alone significantly enhanced the formation of new blood vessels with potencies comparable to $VEGF_{165}$. These results demonstrate the potential for vascular therapy of glycotherapeutic agents targeted at augmenting the bioactivity of $VEGF_{165}$.

1. Introduction

Ischaemic heart and vascular disease including myocardial infarction and stroke remain the leading cause of death worldwide [1], but treatments remain problematical. The use of pro-angiogenic agents to stimulate the formation of new blood vessels, a therapeutic angiogenic approach, is currently being trialed to improve perfusion at ischaemic sites [2–4]. Vascular endothelial growth factor (VEGF) appears particularly promising. The most abundant isoform, $VEGF_{165}$, has excited the most interest because of its powerful physiological effects [5]. However, clinical trials testing recombinant $VEGF_{165}$ have so far been disappointing [5–8], in part due to its instability in physiological environments [9]. Thus, maintaining effective concentrations of $VEGF_{165}$ at ischaemic sites has proven difficult, resulting in high, and often excessive dosing that leads to unwanted side effects such as aberrant angiogenesis [2,5–7]. As such, there is still a pressing need for a safe and effective therapeutic capable of restoring blood supply.

Numerous studies have demonstrated the essential role of heparan sulfate (HS) in mediating $VEGF_{165}$-directed angiogenesis [10,11]. HS is composed of a family of variably sulfated glycosaminoglycans (GAGs) consisting of repeating disaccharide units of glucuronic acid (GlcA) and glucosamine (GlcN) [12,13]. HS binds to the carboxyl-terminal of $VEGF_{165}$, stabilises and enhances the interaction between $VEGF_{165}$ and VEGF receptor 2 (VEGFR2), and so regulates endothelial proliferation, tube formation and vascular hyper-permeability [11,14,15]. As HS can be readily harvested from cultured tissues and cells, it is emerging as a new class of therapeutic compound capable of augmenting blood vessel growth.

Subtle variations in disaccharide sequence, chain length and biosynthesis endow distinct HS variants in each tissue with unique growth-factor binding capacity, and thereby targeted control of downstream bioactivity [16,17]. Maximising the therapeutic potential of HS thus requires a strategy for dealing with this

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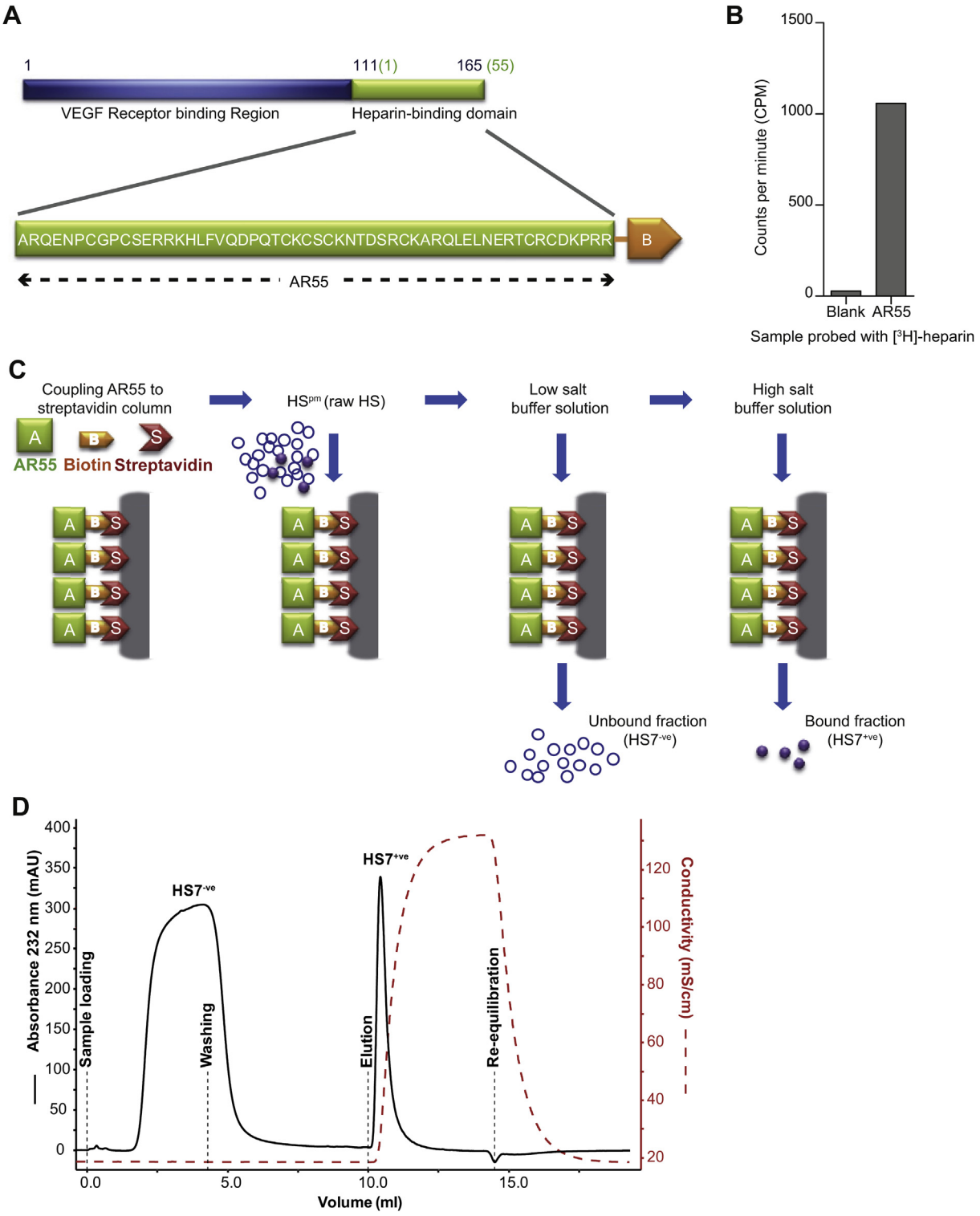

Fig. 1. Purification of $VEGF_{165}$-binding HS. (A) Schematic of $VEGF_{165}$ showing the VEGF receptor binding region 1–110 aa (blue) and the carboxyl-terminal heparin-binding domain 111-165aa (green), the latter of which was synthesised as a 6-aminohexanoic acid-biotin linked peptide for HS purification. The $VEGF_{165}$ heparin-binding domain peptide was labelled AR55. (B) Filter-binding assay of AR55-AHX-biotin immobilised on nitrocellulose membranes and exposed to $[^3H]$-heparin. Data is representative of duplicate experiments. (C) Schematic of $VEGF_{165}$-binding HS ($HS7^{+ve}$) isolation from porcine mucosal HS (HS^{pm}): HS^{pm} was dissolved in low salt buffer (20 mM PBS, 0.15M NaCl, pH 7.2) and loaded onto a streptavidin column coupled with AR55. The column was washed with the same buffer until the baseline reached zero, and $HS7^{+ve}$ was then eluted using high salt buffer (20 mM PBS, 1.5 M NaCl, pH 7.2). Both $HS7^{+ve}$ and unbound HS ($HS7^{-ve}$) were collected. (D) Chromatogram depicting the isolation of $HS7^{+ve}$ from HS^{pm}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)