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# A novel peptide sequence in perlecan domain IV supports cell adhesion, spreading and FAK activation

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**Abstract**

Perlecan/HSPG2 is a large, multi-domain, multifunctional heparan sulfate proteoglycan with a wide tissue distribution. With the exception of its unique domain I, each of perlecan's other four domains shares sequence similarity to other protein families including low density lipoprotein (LDL) receptor, laminin alpha chain, neural cell adhesion molecule (NCAM), immunoglobulin (Ig) superfamily members, and epidermal growth factor (EGF). Previous studies demonstrated that glycosaminoglycan-bearing perlecan domain I supports early chondrogenesis and growth factor delivery. Other sites in the core protein interact with other matrix molecules and support cell adhesion, although the peptide sequences involved remain unidentified. To identify novel functional motifs within perlecan, we used a bioinformatics approach to predict regions likely to be on the exterior of the folded protein. Unique hydrophilic sequences of about 18 amino acids were selected for testing in cell adhesion assays. A novel peptide sequence (TWSKVGGHLRPGIVQSG) from an immunoglobulin (Ig) repeat in domain IV supported rapid cell adhesion, spreading and focal adhesion kinase (FAK) activation when compared to other peptides, a randomly scrambled sequence of the domain IV peptide or a negative control protein. MG-63 human osteosarcoma cells, epithelial cells and multipotent $C_3H10T1/2$ cells, but not bone marrow cells, rapidly, i.e., within 30 min, formed focal adhesions and assembled an actin cytoskeleton on domain IV peptide. Cell lines differentially adhered to the domain IV peptide, suggesting adhesion is receptor specific. Adhesion was divalent cation independent and heparin sensitive, a finding that may explain some previously poorly understood observations obtained with intact perlecan. Collectively, these studies demonstrate the feasibility of using bioinformatics-based strategies to identify novel functional motifs in matrix proteins such as perlecan.


*Keywords:* Perlecan; Immunoglobulin repeat; Cell adhesion; Extracellular matrix; Heparan sulfate proteoglycan; Peptide

## 1. Introduction

Perlecan (HSPG2) is a large heparan sulfate proteoglycan expressed in a wide variety of tissues and tissue structures including cartilage and basal lamina (Hassell et al., 2002). Perlecan has been identified in species ranging from insects and nematodes to mammals (Hassell et al., 2002; Rogalski et al., 2001; Voigt et al., 2002). Severe developmental defects are associated with perlecan mutations in all species examined to date (Arikawa-Hirasawa et al., 1999, 2001; Costell et al., 1999; Nicole et al., 2000; Rogalski et al., 2001; Voigt et al., 2002). Perlecan participates in diverse biological functions including growth factor delivery, promotion of cell adhesion, proliferation and cancer progression (Farach-Carson et al., 2005; Iozzo, 2005; Savore et al., 2005; Yang et al., 2005). This spectrum of functions is reflected by the complexity of the protein core consisting of five distinct, independently functioning domains (Fig. 1). Unique domain I contains three glycosaminoglycan attachment sites as well as an SEA domain (Dolan et al., 1997). The other four domains possess sequence similarity to other protein families (Murdoch et al., 1992; Noonan et al., 1991; Farach-Carson and Carson, 2007). Domain II contains repeat sequences highly similar to LDL-receptor, while domain III is comprised of tandem globular domain repeats similar to domain IV of the laminin alpha (A) chain and containing both laminin EGF-like and laminin G (LG) like repeats. An RGD motif is found in mouse, but not human, perlecan domain III (Chakravarty et al., 1995).

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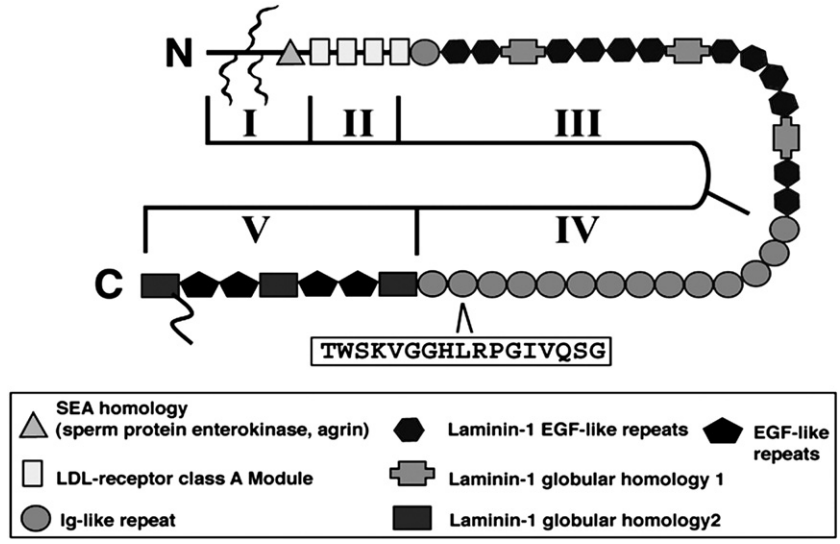


Fig. 1. Domain structure of murine perlecan showing location of perlecan domain IV peptide, TWSKVGGHLRPGIVQSG, in the fourteenth Ig-like repeat of domain IV. The diagram shows a schematic of perlecan indicating the five major domains (I–V) and the amino-(N) and C-(C) termini. Threadlike structures in domains I and V represent attached glycosaminoglycan chains. The location and sequence of the peptide used for most studies in this paper is indicated in the box. Similarities of various motifs in perlecan to other protein motifs are indicated in the inset. This figure was adapted from Olsen (1999).

Domain IV contains repeats similar to those found in the immunoglobulin (Ig) superfamily member, neural cell adhesion molecule (N-CAM) or platelet endothelial cell adhesion molecule (PECAM/CD31). The C-terminal domain V shows sequence similarity to G region of the laminin alpha (A) chain. There also are epidermal growth factor (EGF)-like sequences spaced

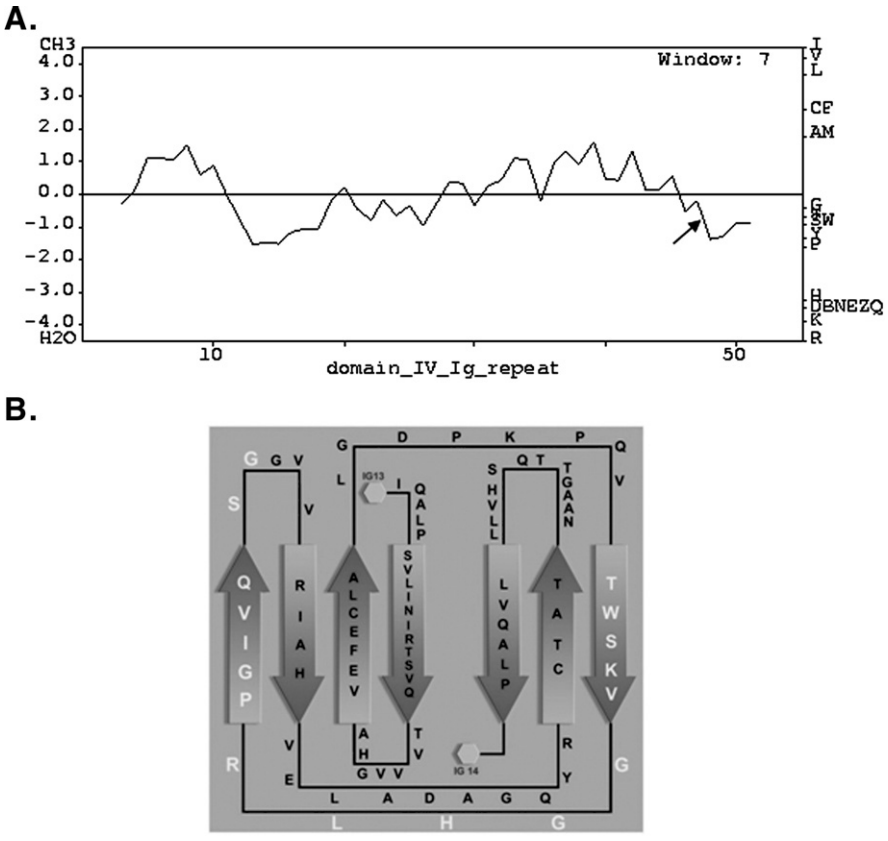


Fig. 2. Hydrophilicity plot and secondary structure prediction of perlecan domain IV Ig module sequence. Hydrophilicity (A) was determined using the online program GREASE from the SDSC Workbench, the arrow indicates the hydrophilic region in which the chosen peptide is located. Secondary structure (B) was predicted based on the homology model of Hopf and colleagues as described in "Experimental procedures". The TWSKVGGHLRPGIVQSG sequence (white letters) is predicted to lie on the outside of the Ig module where it would be in contact with the aqueous solution and potentially interact with other proteins or surfaces.