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ORIGINAL ARTICLE

Recombinant expression and purification of human placental growth factor 1 and specific camel heavy chain polyclonal antibody preparation

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Abstract Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family. Unlike VEGF, PlGF is dispensable for normal cell development as well as playing various roles in pathological angiogenesis which occurs in tissue ischemia, inflammation, and malignancy. The PlGF-1 has been considered as a potential candidate for the diagnosis and targeting of pathological angiogenesis. Camelidae serum contains an important fraction of functional antibodies, called heavy-chain antibodies (HcAbs) that are naturally devoid of light chains. Camelid HcAbs recognize their cognate antigens by a single variable-domain, referred to as VHH or Nanobody.

Here, we describe the expression and purification of recombinant human PlGF-1 (rhPlGF-1). This protein was subsequently used for the preparation of camel heavy chain polyclonal antibody against rhPlGF-1.

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The recombinant expression plasmid pET-26b-hPlGF-1 was introduced into *Escherichia coli* BL21 cells to express the rhPlGF-1 protein. Purified rhPlGF-1 was used to immunize camel, the specific reactivity of HcAb was determined with ELISA and western blot. Western blot analysis indicated that the antiserum specifically reacted to the recombinant protein. The rhPlGF-1 protein and its antibody may be used for the development of detection assays needed for clinical research.

1. Introduction

Angiogenesis is an essential process in fetal development, tissue regeneration and remodeling in pathologic conditions such as tumor invasion and metastasis (Folkman, 2007). Angiogenesis is a complex process which is stimulated by a variety of angiogenic growth factors (Bikfalvi, 2012; Gordon et al., 2010). The vascular endothelial growth factors (VEGFs) are considered as key molecules in this process. The VEGF family currently includes VEGF-A, -B, -C, -D, -E, -F and placental growth factor (Roy et al., 2006). PlGF is a secreted protein that shares substantial structural similarity with VEGF. Four PlGF isoforms (i.e. PlGF-1–4) are produced by alternative splicing of PlGF genes in human.

It has been shown that PlGF-1 can stimulate vessel growth and maturation directly by affecting endothelial and mural cells, and indirectly by recruiting pro-angiogenic cell types, the recruitment and maturation of angiogenesis-competent myeloid progenitor cells and activating macrophages to release angiogenic and lymphangiogenic molecules (Selvaraj et al., 2003). In physiological condition the PlGF is undetectable but in pathological conditions is mainly up regulated. Therefore the blockade of the PlGF can lead to the inhibition of neovascularization and tumor metastasis (Fischer et al., 2008).

Camelidae serum contains an important fraction of functional antibodies, called heavy-chain antibodies (HcAb) which are naturally devoid of light chains. Therefore, the Camelid HcAbs recognize their cognate antigens by a single variable-domain, referred to as VHH or Nanobody (Behdani et al., 2012; Muyldermans et al., 2009). Nanobodies have several inherent, advantageous properties such as strict monomeric behavior, high affinity, solubility and stability and remarkable expression yield of recombinant VHH in bacteria or yeast. These characteristics have made the VHH an attractive next-generation reagent for immunoassays and therapeutic applications.

The aim of this study was the production of rhPlGF-1 and preparation of camel heavy chain polyclonal antibody directed against this protein.

2. Materials and methods

2.1. Construction of pET-26b-hPlGF-1 plasmid

Escherichia coli B21 strain and pET-26b plasmid were purchased from Iranian Gene Bank (Pasteur Institute of Iran, Tehran, Iran).

The synthetic hPlGF-1 construct (BIOMATIK, Canada) was composed of coding sequence of the human PlGF-1 inserted between *Nco*I and *Xho*I restriction sites of the pET-26b plasmid (Fig. 1). This construct was transformed into *E. coli* BL21 cells. The integrity of the final construct (pET-26b-hPlGF-1) and the transformation process were confirmed by restriction digestion and DNA sequencing.

2.2. Expression and purification of rhPlGF-1

The protein expression was induced by the addition of 1 mM isopropyl-D-thiogalactopyranoside (IPTG). After overnight incubation at 37 °C, the bacterial cells were pelleted and homogenized by ultrasonication in analysis buffer (8 M urea, 20 mM Tris–HCl, 500 mM NaCl, 50 mM Imidazol, 0.5% triton X-100 and protease inhibitor). The bacterial cell lysate was centrifuged at 8000*g* for 30 min. The supernatant was loaded on a Ni–NTA column and washed with 4 M urea, 20 mM Tris–HCl, 500 mM NaCl, 60 mM Imidazol, and protease inhibitor. The bound proteins were eluted with 500 mM Imidazole in PBS. The eluted protein was loaded on a gel filtration column (Sephadex 75- GE-Healthcare).

2.3. SDS–PAGE and western blotting analysis

Purified rhPlGF-1was determined on SDS–PAGE with 12% resolving gel and 5% stacking gel. For western blotting the separated protein was transferred onto nitrocellulose membrane, and the membrane was blocked with 2% skimmed milk in PBS for 2 h at room temperature (RT) and incubated with 1/3000 mouse anti-hPlGF-1(Cell Science) overnight at 4 °C.

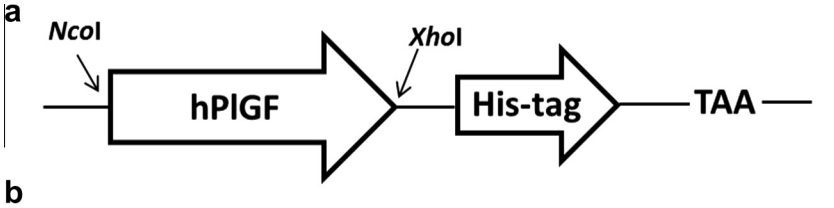

Figure 1 (a) Schematic expression cassette of pET26-hPLGF-1, (b) The amino acid sequence of hPLGF-1.