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Review

Phage library-screening: A powerful approach for generation of targeting-agents specific for normal pancreatic islet-cells and islet-cell carcinoma in vivo

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ABSTRACT

Phage display technology is a powerful approach for the generation of peptides and antibodies that target specific organ- or tumor-structures. By applying this approach to rats in vivo or freshly isolated rat islets in vitro, we have recently reported the successful isolation of internalizing single-chain antibodies (SCA-antibodies), which are highly specific for the endocrine-cells of a pancreatic islet (either beta- or alpha-cells) both in rodents and in humans. Moreover, others have reported peptides targeting specifically the vascular endothelium of normal or pre-malignant islets or advanced islet-cell tumors. The features of these antibodies and peptides are compatible with a potential use for in vivo delivery of molecular cargos (e.g. imaging agents and therapeutics). Therefore, this article reviews the principles of phage display, provides an overview about agents either specific for the endocrine-cells or the vascular endothelium of islets, discusses methododical key elements for the generation of these ligands and highlights remaining questions and potential future perspectives.

Contents

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1. Introduction

Pancreatic beta-cell mass is markedly reduced in patients with both type 1 and type 2 diabetes, most likely as a consequence of an increased rate of beta-cell apoptosis [1,2]. Therefore, strategies for restoring beta-cell function and preserving islet mass and allowing for accurate and non-invasive assessment of the beta-cell mass in humans are critically needed. Such strategies would be greatly aided by the ability to target protective genes, proteins, small molecules or imaging agents that are highly selective and to non-invasively target pancreatic beta-cells in vivo. In recent years, several known islet- or beta-cell specific (or enriched) surface markers (e.g. glucagon-like peptide 1 receptor and sulfonylurea receptor 1), secretory vesicle components (e.g. zinc and vesicular monoamine transporter 2), or transporter molecules (e.g. glucose transporter 2 and glucokinase), have been evaluated for their suitability as potential islet imaging targets. However, as yet none of these targets or tracer probes has allowed for successful islet-targeting in humans in vivo [3–8]. Therefore, in order to target the beta-cells precisely in humans in vivo aiming to increase or to image the endogenous beta-cell mass, novel tools are highly warranted.

Islet-cell carcinoma is a tumor entity with a poor prognosis and presentation is related to the mass effect of the tumor and/or symptoms related to the hormone-excess (e.g. hypoglycaemia) [9]. Surgery remains the primary therapy, however this procedure is often not applicable to patients, because the disease is often already extended at diagnosis. Moreover, chemotherapeutics or radiotherapy is limited due to toxic side-effects. Thus, in patients with islet-cell carcinoma long-term survival is uncommon. Therefore, the availability of agents that are highly specific either for pre-malignant islet-cells or advanced islet-cell tumors may lead to new diagnostic or treatment options e.g. specific delivery of radionuclides for radiotherapy.

One reason for the obvious difficulties in generating such agents is the lack of knowledge about potential targets that are exclusively expressed (or enriched) in normal islet-cells or islet-tumor cells. One way to overcome these challenges is to use phage display [10]. By these means, a number of very promising agents have been developed, and therefore this article provides an overview about principles of this technology, the agents and their targets and discusses remaining questions and future directions.

2. Phage display technology

Bacteriophage (termed phage), are single-stranded DNA viruses that infect various gram-negative bacteria, including *E. coli*. The phage display technology was first introduced in 1985 by George Smith [11], and since then, a large number of phage libraries expressing proteins (e.g. antibodies and peptides) have been constructed [12–16]. The encoded proteins (e.g. antibodies and peptides) are expressed ("displayed") on the phage surface as a fusion product with one of the phage coat proteins (Fig. 1). This is achieved by the introduction of defined exogenous protein sequences into a location in the genome of the phage capsid proteins. By applying this technology, phage display libraries containing simultaneously up to 10^{10} different proteins have been easily developed.

3. Biopanning

A phage library displaying proteins (e.g. peptides and antibodies) of a known diversity (up to 10^{10}) is screened against immobilized target-proteins in vitro, soluble cells in vitro or injected in animals or humans in vivo [17,18]. Phages that bind to a target structure (e.g. organ, specialized region within an organ, and tumor cells) are then eluted and easily amplified by growing in bacteria (Fig. 2). This so called "biopanning" is repeated several times (normally at least three rounds) to enrich the population of phage that binds best to the target structure. Subsequently, the displayed proteins are identified by DNA sequencing of these phage clones. Finally, monoclonal clones expressing a specific protein are selected and then tested for their targeting purposes in vitro and/or in vivo.

Since target-cell specific delivery requires mostly removal of the protein from the context of the phage, soluble peptides or antibodies can easily be produced in culture. For this purpose, bacteria (e.g. HB2151) are infected with a selected phage clone and grown in an appropriate medium. Subsequently, isopropyl β-D-thiogalactoside is added to induce protein expression and the supernatant containing the protein of interest is then purified e.g. by metal affinity chromatography. Finally, the protein is checked for purity e.g. by SDS-gel electrophoresis and western blotting.

4. Approaches and ligands

By applying phage display screening, novel ligands binding to the vascular endothelium or the endocrine-cells of pancreatic islets have

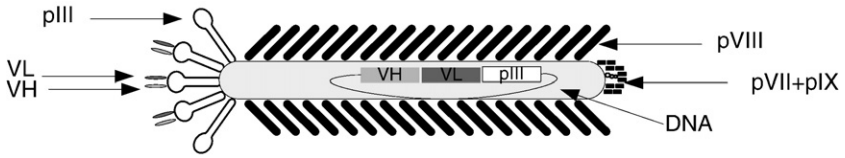

Fig. 1. Schematic structure of a phage displaying multiple copies of a single-chain antibody (VH = variable heavy chain, VL = variable light chain). The information for the single-chain antibody was introduced in a location of the genome for the phage coat protein pIII, and thus the antibodies are displayed fused to the pIII coat protein on the phage surface. An alternative startegy would be to express the single-chain antibodies with the other coat proteins of the phage pVIII or pVII + pIX.