The VP1u of B19V

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The viral protein 1 unique region (VP1u) of human parvovirus B19 (B19V) is a multifunctional capsid protein with essential roles in virus tropism, uptake, and subcellular trafficking. Besides the essential functions in B19V infection, the remarkable erythroid specificity of the VP1u makes it a unique erythroid cell surface biomarker. Moreover, the demonstrated capacity of the VP1u to deliver diverse cargo specifically to cells around the proerythroblast differentiation stage, including erythroleukemic cells, offers novel therapeutic opportunities for erythroid-specific drug delivery.

Keywords: Parvovirus ; B19V ; VP1u ; Receptor ; Drug delivery

1. Introduction

The *Parvoviridae* is a family of nonenveloped viruses that packages a linear, single-stranded DNA genome (~5 kb) within a small (~25 nm) icosahedral capsid. As a direct consequence of their limited coding potential, parvoviruses are particularly dependent on host cellular factors for their replication [1][2]. Parvoviruses are widely spread in nature and their host range might span the entire animal kingdom [3]. Depending on their host, members of the family *Parvoviridae* are subdivided into the subfamilies *Parvovirinae*, infecting vertebrates and *Densovirinae*, infecting insects and other arthropods. Viruses that infect vertebrates, including humans, are further divided into the dependoparvoviruses and the autonomous parvoviruses [4]. The dependoparvoviruses replicate only in the presence of a helper virus, such as adenovirus or herpesvirus. The adeno-associated viruses (AAVs) are not linked with any known pathology, have a wide tissue specificity, and replicate in dividing and nondividing cells. These properties make AAVs useful gene transfer vehicles for therapeutic applications [5]. Although autonomous parvoviruses use similar strategies for cell entry and replication, they differ substantially in their pathogenic potential, which ranges from subclinical to severe or even lethal infections [2]. As autonomous parvoviruses can only replicate in dividing cells, when the host cell DNA replication machinery becomes available, they tend to cause more severe infections in young than in adult hosts.

While most ssDNA viruses show a circular genome structure, parvoviruses have a linear genome, that is typically organized in two open reading frames (ORFs). The ORFs are flanked by palindromic sequences of variable length, which fold into hairpin structures and are essential for replication [6][7]. The 5′ ORF (ns or rep gene) encodes for the regulatory nonstructural protein(s) required for viral DNA replication and packaging. The 3′ ORF (cap gene) encodes two to four variants of a single capsid protein (VP). Following a principle of genetic economy, the different VPs are generated by alternative splicing or alternative codon usage, but also by post-translational proteolytic processing during entry, resulting in a common C-terminal sequence but different N-terminal extensions of variable length [8][9][10]. The T = 1 icosahedral parvovirus capsid is assembled from 60 VPs, however, the number of N-terminal VP variants used to assemble the infectious particles varies from two (VP1 and VP2) to four (VP1–VP4) depending on the genus. The VP variants are numbered in order of length, with VP1 being the largest variant. The common C-terminal region of the VPs forms the capsid shell, which consists of a conserved alpha-helix and a jelly roll motif containing eight antiparallel β-strands. The different configurations of the loops connecting the conserved β-strands delineate the surface topology, which is characteristic to each parvovirus genus and define the virus tropism and antigenicity [11]. Despite low sequence identity, the parvovirus capsids display structural features that are conserved across different genera, i.e., a narrow depression at the twofold axis of symmetry, protrusions of variable size and shape at the threefold axis and a canyon-like structure encircling a cylindrical pore at the fivefold axis connecting with the interior of the capsid.

2. Human Erythroparvovirus B19 (B19V)

B19V is the most prominent and well-characterized human pathogen within the *Parvoviridae* causing a mild childhood rash disease named *erythema infectiosum* or fifth disease [12]. The infection is often asymptomatic; however, in adults, B19V infection may induce a wide range of more severe pathological conditions, such as arthralgias and arthritis [13]. B19V infection may lead to aplastic crisis in patients with pre-existing bone marrow disorders and shortened red cell

survival [14] and persistent infection in immunocompromised persons. Infection during pregnancy may result in *hydrops fetalis* and fetal death [15]. B19V was the first parvovirus known to cause disease in humans [16]. Since 2005, other human parvoviruses have been identified and include human bocavirus (HBoV1-4), parvovirus 4, bufavirus, tusavirus and cutavirus. Except for HBoV, which has been implicated in acute respiratory tract infections[17], the rest are emergent human parvoviruses with uncertain clinical significance [12][18].

B19V is transmitted via aerosol droplets that come into contact with the upper respiratory tract mucosa [14]. The virus crosses the mucosal epithelium through a yet unknown mechanism and disseminates with the bloodstream to the bone marrow, where it infects erythroid precursors at a particular erythropoietin (EPO)-dependent stage of differentiation [19][20][21]. The extraordinary narrow tropism of B19V is mediated at different levels of the viral life cycle. Crucial steps of the viral infection, such as uptake, genome replication, transcription, splicing and packaging, are restricted to the EPO-dependent erythroid differentiation around the proerythroblast stage [21][22][23][24][25][26][27]. The lytic replication cycle results in the destruction of the erythroid precursor cells [28][29], which accounts for the hematological syndromes observed during the infection [14]. Acute infection frequently results in high-titer viremia, which precedes the onset of clinical manifestations and has been associated with B19V transmission through transfusion and plasma-derived medicinal products [30].

3. Biotechnological Applications of the VP1u of B19V

Nanocarriers are designed to efficiently deliver therapeutic molecules to specific tissues minimizing adverse effects [31]. Despite important progress, the drug delivery technology based on synthetic nanocarriers remains highly inefficient. One meta-analysis revealed that over 99% of the drugs do not reach the diseased cells and accumulate instead in non-target tissues or are cleared from the body [32]. Ideally, nanocarriers must specifically internalize into the target cells, escape from the endocytic compartment, and release their payload into the cytosol. These processes resemble the early infection steps of viruses, which operate as powerful natural nanocarriers to efficiently deliver genetic material into target cells by complex mechanisms shaped by evolution. The targeting machinery that is engaged in the early viral infection steps can be utilized to generate virus-inspired nanocarriers as efficient drug or gene delivery vehicles [33][34]. In this regard, the VP1u of B19V includes many interesting features that can potentially be exploited for drug delivery and diagnostics, i.e., specific cell targeting, efficient cell entry, and endosomal escape.

3.1. Specific Biomarker for EPO-Dependent Erythroid Differentiation Stages

Diverse hematological conditions (e.g., leukemia, thalassemic and myelodysplastic syndromes, bone marrow metastases of solid tumors, septicemia, or severe health conditions after surgery) are typically associated with the presence of erythroblasts outside the bone marrow [35][36][37][38][39][40]. Accordingly, the screening of peripheral blood for nucleated red blood cells (NRBCs) is used to recognize hematological disorders or severe health conditions. Assays to detect NRBCs must be very sensitive because the presence of only a few NRBCs can indicate serious underlying disorders. Unfortunately, automated hematology analyzers may not detect low levels of NRBCs. Besides, they generate suspect flags, which should be examined manually [41]. The currently used automated detection of NRBCs in peripheral blood has a detection limit of 1-2 erythroblasts per 100 white blood cells [42]. In comparison, VP1u decorated MS2 capsids were able to detect as few as one erythroleukemic UT7/Epo cell in 100,000 isolated white blood cells (unpublished observations). The sensitive identification of erythroblasts in the peripheral blood by fluorescent VP1u bioconjugates has the potential to improve the detection of diverse hematological disorders or severe health conditions and to facilitate an early diagnosis without the systematic need of an invasive technique such as bone marrow biopsy.

The precise identification and isolation of erythroid progenitor cells is important in hematological research and in diagnostics to characterize and treat bone marrow disorders. However, the technique remains rather complex and laborious, since the currently used markers are not lineage-specific (CD36, CD38, CD44, CD45, CD71, CD105, EPOR) or are broadly expressed during the erythroid development (glycophorin A). Therefore, the combination of several antibodies is necessary to achieve the correct identification [38][43][44][45][46][47][48]. In contrast, the fluorescent VP1u bioconjugate appeared as a unique and highly sensitive marker for the EPO-dependent erythroid differentiation stages and readily detected these cells in heterogeneous cell populations from different tissues [21]. The findings show the potential of the VP1u as a biomarker to identify and sort erythroid differentiation stages in a simpler procedure than it has been practiced so far.

It is expected that the future biotechnological applications of the VP1u will be spurred by the identification of its cognate receptor. However, the identity of the VP1u receptor will not necessarily be determinant for the applicability of the VP1u as a specific cellular marker. Historically, it is not uncommon to use cell surface markers to identify cell populations based on empirical evidence without knowing the identity and/or the function of the targeted receptors.

3.2. Specific Drug Delivery and Chemotherapy

3.2.1. β-Hemoglobin Disorders

β-hemoglobin disorders are a group of highly prevalent hereditary diseases caused by mutations in the gene encoding for the β-chain of hemoglobin, resulting in qualitative and quantitative defects in β-globin production. β-thalassemias are a heterogeneous group of genetic disorders characterized by the partial or complete absence of β-globin chain production, leading to anemia and iron overload. The disease is highly prevalent with 80–90 million carriers worldwide. Without diagnosis and appropriate treatment, the severe forms of β-thalassemia lead to death before age 20 [49]. Sickle cell disease (SCD) is the most common and severe hemoglobinopathy. In SCD, a single mutation in the β-globin gene results in the production of an aberrant hemoglobin molecule, which causes the rigid sickle-like shape of erythrocytes. Without treatment, SCD is lethal before age five [50].

Patients with severe β-hemoglobin disorders require regular blood transfusions, which lead to iron overload and related complications. Accordingly, iron chelation therapies are also required [51][52]. The most severe forms of the disease have been successfully treated by allogeneic hematopoietic stem cell transplantation from a matched related donor. However, major drawbacks are the difficulty to find a histocompatible donor and the need for extensive immunosuppressive regimens, with the risk of immunological complication. Besides, this approach is not accessible for many affected individuals [53][54]. Gene therapy and gene editing strategies to restore the globin genes have generated promising results. However, these approaches lack cell-specific vectors, resulting in poor efficiency and the risk of insertional oncogenesis [55][56][57].

Due to the numerous drawbacks associated with the current therapeutic strategies, there is a great interest in developing novel therapeutic options. The therapeutic targeting of RNA by double-stranded RNA-mediated interference (RNAi) or by antisense oligonucleotides (ASOs) allows specific inhibition of the target of interest and a very rapid transferability to the clinics [58]. However, the delivery of nucleic acid molecules to the bone marrow remains highly inefficient. The MS2 capsid is a well-studied vector for drug delivery and can be easily loaded with therapeutic ASOs or small interfering RNAs (siRNA) [59][60]. This strategy provides protection of the therapeutic nucleic acid molecules in the extracellular milieu, avoids solubility problems, and thus allows more options to improve the modifications of the oligonucleotides. In a previous study, we showed that anchoring of VP1u subunits to the surface of MS2 capsids retargets the particles to erythroid cells. This finding offers the opportunity to deliver encapsidated genetic material specifically to this cell population [26]. Potential targets of therapeutic ASOs or siRNA might be different factors involved in the regulation of erythropoiesis, such as transferrin receptor 2, or regulatory elements of fetal hemoglobin, such as B-cell lymphoma/leukemia 11A and erythroid Kruppel-like factor. Specific downregulation of such factors in erythroid progenitor cells would significantly alleviate symptoms of β-hemoglobin disorders [61][62][63][64].

3.2.2. Erythroleukemia

Acute erythroleukemia is a rare disorder associated with a poor prognosis. A study reported a median overall survival of 8 months [65]. The treatment of erythroleukemia is compromised due to the systemic distribution and resistance of the malignant cells to chemotherapeutics [66][67]. Therefore, the successful elimination of erythroleukemic cells by a cytotoxin requires a “magic bullet” strategy—an efficient and specific targeting of the toxin to cancer cells—minimizing adverse effects to the surrounding healthy cells [68]. Erythroleukemias exhibit proliferating cancer cells in the early and intermediate erythroid differentiation stages [69], which are the target cells of the VP1u. Accordingly, the VP1u-mediated toxin delivery represents a possible strategy to overcome the resistance of erythroleukemia to chemotherapeutics. In previous studies, VP1u successfully targeted a toxin specifically to malignant erythroid precursors and thus selectively eliminated these cells from a mixed cell culture [70].

The immunity of many individuals against B19V would represent a serious obstacle for the application of the VP1u-targeted delivery. About half of the human population is seropositive for anti-B19V antibodies. Similar problems are faced in the application of AAV vectors for gene therapy, where many individuals have antibodies against serotypes 2 and 3 [71]. Following the natural mechanism of viruses to evade the immune system, the AAV researchers are searching for AAV isolates and isotypes, which are not neutralized by the common pool of antibodies, but still offer the beneficial properties of the original virus [72][73][74]. In the case of a short protein with a single function as with the RBD of the VP1u, an immune escape by antigenic drift is easier to achieve without disturbing the receptor binding and internalization capacity. The natural mutations observed in various B19V isolates together with the mutational studies already performed [75], provide an excellent basis to mimic an antigenic drift of the VP1u RBD without decreasing the targeting function of the protein. Furthermore, there exist different options to reduce the antigenicity of a therapeutic protein, such as a fusion with an abundant endogenous protein as serum albumin or the immunoglobulin constant fragments [76][77][78]. The coupling to these endogenous proteins does not only circumvent the immune response, but also considerably increases the solubility,

stability, and serum half-life of the therapeutic proteins. In line with this concept, bovine serum albumin (BSA) was used as an adaptor molecule for the attachment of the toxins to a VP1u-NeutrAvidin complex. The results showed that the modified BSA remained soluble after the attachment of 20–30 fluorescein or toxin molecules to the protein and was targeted exclusively to VP1u-expressing cells. The stability of the drug attachment might be increased by packing the effector molecule into a capsid, as shown with the MS2 bacteriophage in previous studies [59][60][79]. The specific delivery of an encapsidated effector allows a higher dose per delivered particle without increasing toxicity. Besides, the capsid can be engineered to incorporate multiple residues to improve the targeting efficiency.

4. Concluding Remarks

The VP1u is a key component of the capsid of human parvovirus B19 with essential functions in multiple steps of the infection, such as tissue tropism, uptake, intracellular trafficking, and entry. The VP1u is also the immunodominant region of the capsid and a crucial component for prospective vaccines. In the future, efforts will be focused to better understand the essential functions of VP1u in B19V infection and to identify the VP1u interactome, notably its cognate cell receptor.

Recent innovations in protein engineering and nanomaterials science have the potential to revolutionize the conventional methods of diagnosis and treatment, bringing new hopes to patients. However, to date, a major barrier in their clinical application remains their poor selective targeting. Only a few clinically approved nanoscale delivery vehicles integrate molecules to selectively target the cargo to the tissue of interest. In this regard, the remarkable erythroid specificity of the VP1u offers novel opportunities to generate virus-inspired biomarkers and nanocarriers to specifically target erythroid cells. This approach may contribute to a better understanding of the mechanisms governing erythroid development and to treat disorders of the erythroid lineage. Efforts to circumvent the VP1u immune response and to optimize the stability and density of cargo delivery will facilitate its transferability to human diagnostics and therapies.

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