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Cellular targets for improved manufacturing of virus-based biopharmaceuticals in animal cells

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The past decade witnessed the entry into the market of new virus-based biopharmaceuticals produced in animal cells such as oncolytic vectors, virus-like particle vaccines, and gene transfer vectors. Therefore, increased attention and investment to optimize cell culture processes towards enhanced manufacturing of these bioproducts is anticipated. Herein, we review key findings on virus–host interactions that have been explored in cell culture optimization. Approaches supporting improved productivity or quality of vector preparations are discussed, mainly focusing on medium design and genetic manipulation. This review provides an integrated outline for current and future efforts in exploring cellular targets for the optimization of cell culture manufacturing of virus-based biopharmaceuticals.

Virus-based biopharmaceuticals produced in animal cells

Virus-based biopharmaceuticals (VBBs) comprise several bioproducts used for vaccination and gene transfer (Box 1). Vaccines have traditionally received more attention, but the interest in viral vectors has also been growing, leveraged by the recent marketing authorization for the first gene therapy medicine granted by the European Commission [1]. Therefore, active investment in the field can be anticipated, including optimizing VBB manufacturing. Herein, we revisit key findings on the interactions of animal viruses and their hosts, and discuss the main cell culture approaches exploring these interactions to improve the production of VBBs.

Many VBBs are produced in bacteria, yeast, or plant cells [2]. However, animal cells gained relevance for VBB manufacturing because they provide important traits difficult to achieve with alternative hosts. For instance, the inability of non-animal cells to support complex post-translational modifications (PTMs) often impairs the capacity of the viral particles to transduce the target cells, particularly relevant for gene therapy, oncolytic virotherapy, and vaccine vectors. Even when transduction is not an issue, animal cells can offer a superior production platform: virus-like particles (VLPs) of hepatitis B virus produced in mammalian cells, although similar in composition, glycosylation, and lipid content, were larger in size and presented increased content of monomers exhibiting higher immunogenicity than the VLPs produced in yeast [2]. The advantages are not restricted to complex PTMs. For instance, HIV-based vectors have only been successfully produced in human and (non-human) primate cells. Alternative hosts, including insect cells, produce high titers of HIV–VLPs, but have failed to produce infectious virions due to abnormal activity of the lentiviral protease [3]. In general, animal cells, and in some cases only those resembling the natural host, allow for robust production of animal viruses and their recombinant derivatives. Here, we discuss how the understanding of virus–host interactions can unveil cellular targets to optimize animal cell culture manufacturing of VBBs.

Virus–host interactions that affect cellular processes

Lipid metabolism

Viruses use lipid scaffolds as physical support for the concentration of viral and cellular factors/proteins during replication and assembly [4]. This reliance often imposes changes to the host lipid metabolism. Increased biosynthesis of fatty acids and phospholipids has been shown to occur after infection of several viruses [5–7], while the inhibition of these pathways frequently impairs viral replication or leads to defective particle formation both for enveloped [8] and non-enveloped viruses [9]. Increased biosynthesis can occur through: (i) higher expression and/or activation of the respective enzymes [8]; (ii) recruitment of biosynthesis-related transcription factors [10]; or (iii) by reducing lipid oxidation [11]. There are also viruses inducing downregulated biosynthesis, counterbalanced with the upregulation of lipid uptake – mainly cholesterol – as in the cases of hepatitis C virus (HCV) [6,12] and murine leukemia virus (MLV) [13].

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Box 1. The toolbox of virus-based biopharmaceuticals

VBBs can be defined as any virus-derived component or virus-based particle with therapeutic or prophylactic application (Figure I). This definition includes viral vectors for gene therapy and oncolytic virotherapy, VLPs, vaccine vectors, inactivated (or attenuated) viruses, and also free viral proteins. The latter are, however, outside of the scope of this review. Viral vectors for gene therapy are recombinant viral particles designed to transfer genetic material into the patient cells to treat or prevent a disease. Oncolytic virotherapy appears as a subfield of cancer gene therapy, typically using replicative competent lytic viruses to infect (and lyse) cancer cells. Viral particles for vaccination can be conceptually divided into three groups: attenuated (or inactivated) viruses (wild type or recombinant versions), VLPs, and vaccine vectors. VLPs are multiprotein structures assembled into a virus-resembling architecture mimicking the structural conformation of native viruses, thereby used for antigen display, but lacking viral genome. Vaccine vectors (or vectored vaccines) can be conceived as frontier particles between VLPs and viral vectors, typically coupling antigen display with nucleic acid delivery to raise both antibody and cytotoxic T cell (CTL) responses.

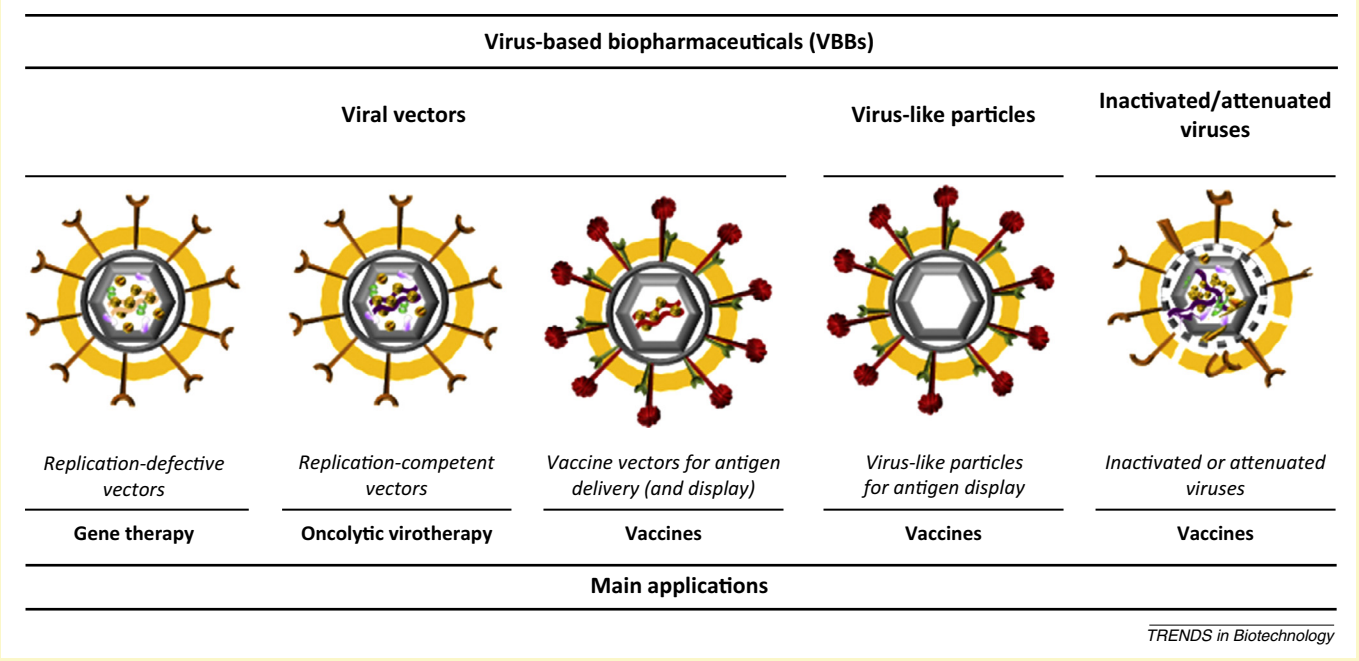

Figure I. Categorization of virus-based biopharmaceuticals.

Energy metabolism

Three main mechanisms – not necessarily independent – by which viruses usurp the host energy machinery have been described: (i) adenosine-5′-monophosphate-activated protein kinase (AMPK) interaction [14]; (ii) Warburg effect-like metabolic reprogramming [15]; and (iii) mobilization of lipid storages [16]. AMPK activation occurs in response to ATP depletion and stimulates catabolic routes, such as lipid oxidation and glucose uptake, and has been described following the infection of a few viruses [17,18]. Yet, not all viruses lead to AMPK activation and the interactions of viruses with AMPK may depend on the type of infection: acute, predominantly associated with activation, or chronic/latent, related to AMPK inhibition [14]. Metabolic reprogramming is usually based on higher expression or activity of the glycolytic enzymes, namely phosphofructokinase, and used to rapidly increase the ATP content [7,19,20]. Mobilization of the triacylglycerol storages and enhanced lipid β-oxidation has also been described [21].

Nucleic acid metabolism and pentose phosphate pathway

Virus replication strongly affects the nucleic acid metabolism of the cell host, ranging from increasing total RNA (or DNA) synthesis and substantial *de novo* nucleotide biosynthesis [20] to arresting the synthesis and hijacking the available nucleic acid content [22]. Increased biosynthesis seems to be predominant in latent/chronic viruses, while arresting is associated with lytic and acute infections. The changes in nucleic acid metabolism have been related to increased enzyme activity and/or expression of nucleotide biosynthesis preceding routes such as the pentose phosphate pathway (PPP) [23]. Alterations in the pool of PPP metabolites have also been described, including elevated levels of ribose-5-phosphate and ribulose-5-phosphate [24].

Oxidative stress metabolism

Viral infections can raise the oxidative burden, mediated by reactive oxygen and nitrogen species [25]. This usually occurs via the inhibition of antioxidant enzymes and the activation of pathways generating oxidants. Some aspects on how the oxidative burden relates to the viral pathogenesis have also been highlighted, including increased viral genome heterogeneity and escape from intracellular antiviral defenses. Several studies suggest that the oxidative environment is a nursing milieu for viral replication. This conclusion may, however, not be straightforward, because some viruses that generated high oxidative load were also shown to upregulate the expression of antioxidant enzymes during the infection