HEK293 Cell Line

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The HEK293 cell line has earned its place as a producer of biotherapeutics. In addition to its ease of growth in serum-free suspension culture and its amenability to transfection, this cell line's most important attribute is its human origin, which makes it suitable to produce biologics intended for human use. At the present time, the growth and production properties of the HEK293 cell line are inferior to those of non-human cell lines, such as the Chinese hamster ovary (CHO) and the murine myeloma NSO cell lines. However, the modification of genes involved in cellular processes, such as cell proliferation, apoptosis, metabolism, glycosylation, secretion, and protein folding, in addition to bioprocess, media, and vector optimization, have greatly improved the performance of this cell line.

Keywords: HEK293 ; recombinant protein production ; cell line engineering

1. Introduction

The human embryonic kidney cell line (HEK293) was created by transforming human embryonic kidney (HEK) cells with sheared fragments of adenovirus type 5 (Ad5) DNA, immortalizing it. Seventeen percent of the extreme left hand of the Ad5 genome is believed to have been integrated into chromosome 19 of the transformed HEK cells [1]. Since its creation, this cell line has been widely characterized and numerous derivative cell lines possessing unique characteristics have been developed. These cell lines include HEK293T [2], HEK293E [3], HEK293-6E [4], HEK293F and its derivatives HEK293FT and HEK293FTM [2][5][6][7], HEK293S [2], and HEK293H [8]. These derivatives and their properties are summarized in **Table 1**.

Table 1. HEK293 cell line derivatives.

HEK 293 Variant	Derivation	Commercially Available?	Desired Characteristic(s)	Year of Derivation
HEK293	Transformation of Human embryonic kidney cells with sheared fragments of adenovirus type 5 (Ad5) DNA, selected for immortalization.	Yes	Parental HEK293 cell line	1973
HEK293S	Adapted for suspension growth	Yes	Grows in suspension in modified minimal Eagle's medium	1984
HEK293T	Stable transfection of the HEK 293 cell line with a plasmid encoding a temperature-sensitive mutant of the SV40 large T antigen	Yes	Amplification of vectors containing the SV40 ori, considerably increasing the protein expression levels during transient transfection.	Before 1985
HEK293FT	Fast-growing variant of HEK293T. Expresses the SV40 large T antigen from the pCMVSPORT6TAg.neo plasmid.		Designed for lentiviral production.	
HEK293F	Cloned from the HEK293 cell line and adapted to commercial medium	Yes	Fast growth and high transfectivity. Growth in chemically defined medium	2014
HEK293H	Cloned from HEK293 to select a clone with good adherence during plaque assays. Later adapted to growth in serum-free medium (SFM) (GIBCO 293H)	Yes	Fast growth in SFM, good adherence during plaque assays, superior transfection efficiencies and a high level of protein expression	1993

HEK 293 Variant	Derivation	Commercially Available?	Desired Characteristic(s)	Year of Derivation
HEK293E(EBNA)	**Expresses the EBNA-1 protein for episomal replication of oriP harboring plasmids**	**Yes**	**Amplification of vectors containing oriP, considerably increasing the protein expression levels during transient transfection.**	
HEK2936E	**This cell line is transfected with EBNA1t, a truncated version of EBV EBNA1 lacking the Gly-Gly-Ala repeats region**		**Has an enhanced ability to produce recombinant protein compared to HEK293-EBNA**	
HEK293FTM	**Derived from 293 cells by stable transfection of an FRT-site containing plasmid and of a TetR expression plasmid.**		**Used for fast and easy generation of a stably transfected cell pool by co-transfecting a Flp-InTM expression vector containing a gene of interest and a Flp recombinase expression vector.**	**Before 2001**
HEK293SG	**Ricin toxin-resistant clone derived from HEK293S by ethylmethanesulfonate (EMS).**	**Yes**	**Lacks N-acetylglucosaminyltransferase I activity (encoded by the *MGAT1* gene) and accordingly predominantly modifies glycoproteins with the Man5GlcNAc2 N-glycan. HEK293SG is used to produce homogenously N-glycosylated proteins**	**2001-2002**
HEK293SGGD (Glycodelete)	**Derives from 293SG through expression of a Golgi targeted form of endoT, an endoglycosidase from the fungus *Trichoderma reesei***		**HEK293SGGD is mainly used to produce proteins for glycosylation studies and structural analysis**	**2010**
HEK293A	**Subclone of the HEK293 cells with a relatively flat morphology**		**Facilitates the initial production, amplification and titering of replication-incompetent adenovirus**	
HEK293MSR	**Genetically engineered from HEK293 to express the human macrophage scavenger receptor**		**Strongly adheres to standard tissue culture plates for dependable results**	

Data adapted from [9][10].

Until two decades ago, the HEK293 cell line was mainly used for transient, small to medium-scale production of research-grade proteins for scientific and pre-clinical studies. Its desirable characteristics have propelled this cell line into a potential producer of biopharmaceuticals [11][12]. These characteristics include suspension growth in serum-free media that enables large-scale production, reproducibility across different batches, rapid reproduction, amenability to different transfection methods, high efficiency of protein production, and—most importantly—its human origin. Five therapeutic proteins produced in HEK293 cells had been approved by the European Medicines Agency or the U.S. Food and Drug Administration for use in humans as of 2015 [13]. This cell line has also been useful in viral vector production as a result of adenoviral DNA in its genome. It has been employed in the production of the influenza virus [14][15], recombinant adenovirus [16], and lentiviruses [17][18]. This cell line is also the preferred host for membrane protein production, where it is most useful as a transient transfection tool for the evaluation of pharmacological properties of multiple receptor subtypes [12][19][20].

The HEK293 cell line is of epithelial origin and adherent in nature. It has been adapted to grow in suspension culture in serum-free or chemically defined media and is currently being utilized for recombinant proteins expression in both adherent and suspension platforms. Adherent platforms include roller bottles [21][22][23], multilayered culture systems (e.g., Nunc Cell Factories, Corning CellStacks and Greiner BIO-ONE CELLdisc™) [24][25][26][27][28], and fixed-bed bioreactors [27][29]. Suspension platforms include batch, fed batch and continuous processes in bioreactors.

Due to its human origin, HEK293 is better suited for production of biotherapeutics for human use since it produces protein with native post-translational modifications (PTMs). Non-human cell lines, such as the Chinese Hamster ovary cells (CHO), have been shown to implement incomplete humanized PTMs and can introduce potentially immunogenic non-human glycosylation patterns, most notably α-gal and NGNA [30][31]. PTMs, especially glycosylation patterns, affect the pharmacokinetics and pharmacodynamics of recombinant biotherapeutics. Although extensive comparative analyses of the PTMs on recombinant proteins produced in numerous cell lines have concluded that the suitability of a cell line for producing human therapeutic proteins should be determined on a case-by case basis [32][33], the HEK293 cell line has

been shown to be especially efficient in tyrosine sulfation and glutamic acid γ-carboxylation when compared to other cell lines [34][35][36][37][38]. The impact of the host cell line on glycan profile has been comprehensively reviewed by Goh and Ng [39]. However, clinical experience with the HEK 293 cell line is not as extensive as for non-human other cell lines, although experience is growing, and there is potential susceptibility to human viral contamination due to the absence of species barrier.

Other human cell lines such as the HT-1080, produced from a fibrosarcoma with an epithelial-like phenotype [11] and the PER.C6 cell line created from human embryonic retinal cells, immortalized via transfection with the adenovirus E1 gene [40], have comparable growth, transfectability, production and safety profiles to the HEK293 cell line and produce complex human proteins with humanized PTMS [13]. However, the HEK293 cell line was used more extensively in different research topics and has numerous derivative cell lines which greatly expands the repertoire of recombinant proteins that it can efficiently produce.

Process and media parameters, such as expression vector, growth media, transfection agent and culture conditions, have been optimized to improved recombinant protein yields from HEK293. Notable innovative approaches include 3D collagen microsphere culture system [41]; the establishment of a glutamine-ammonia ligase (GLUL) mediated gene selection system [42]; glutamine synthase (GS) mediated selection/amplification system, and a dihydrofolate reductase (DHFR) amplification system [43][44], synonymous with the CHO system [45]. Growth and media optimization have led to the development of several formulations of chemically defined growth media and animal-derived component free media additives tailored to HEK293 cell growth and protein expression [46]. Some of the most widely utilized serum-free chemically defined media include ThermoFisher's FreeStyle™ 293, CD 293, 293 SFM II and Expi293™ Expression Medium, Millipore Sigma's EX-CELL® 293 Serum-Free Medium for HEK 293 Cells, and Cytiva's HyClone SFM4Transfx-293 media.

Several transfection reagents have been optimized for use in transfection of HEK293 with plasmid DNA. For example, the Lipofectamine 3000 reagent by Thermo Fisher Scientific is the superior—but cost-prohibitive—option [47], while polyethylenimines (PEI) is the more cost-effective option [48]. Expression vector optimization identified the human cytomegalovirus promoter (CMV) as the most effective promoter for the expression of heterologous proteins in this cell line [49]. Culture parameters, including temperature and carbon dioxide concentration, [50] have also been optimized. Cytostatic agents such as sodium butyrate, trichostatin A, valproic acid, and dimethyl sulfoxide [51], have been used to enhance production. The implementation of these strategies to boost protein yields has resulted in product yields of up to 100–200mg/L in adherent systems, while suspension systems have yielded up to 140–600mg/L of recombinant protein [52] [53].

Yet, despite these significant improvements in recombinant protein and virus production, this cell line continues to lag behind the CHO cell line in growth capacity (Maximal cell density of 3-5 × 10^6/mL for HEK293 vs. 1-2 × 10^7 for CHO) [42], cultivation time (HEK293 doubling time 33 h vs. 14-17 h for CHO), and product yield in stable production systems- up to 4g/L for CHO cells vs. 600mg/L for HEK 293 cells [54]. Consequently, some innate characteristics of HEK293 cells have been modified, resulting in higher recombinant protein yields, efficient nutrient utilization, and more homogenous glycosylation.

2. Affecting HEK293 Cell Growth and Production Performance by Modifying the Expression of Specific Genes

Although the HEK293 cell line trails non-human cell lines such as CHO and NSO, in recombinant protein production and culture performance [13], it is our opinion that this cell line has a place in the production of specific bioproducts. These cells, in addition to their ability to create native PTMs, efficiently execute tyrosine sulfation and gamma carboxylation which makes them the preferred host for recombinant clotting factors and natural anticoagulants production [13]. The cells also gained regulatory approval and have a place as producer of difficult-to-express antibody fragments or artificial scaffolds, which are forecasted to be the future of antibody therapy [55]. In addition, their use in production of gene therapy vectors will only increase as shown by the number of studies and resources directed towards understanding and improving gene therapy vector production in recent years [56]. The author's opinion is that rational engineering strategies targeting bottlenecks in proliferation, carbon metabolism, protein processing and maturation with discovery of engineering targets using functional genomics tools have and will continue to enhance the production efficiency of the HEK293 cell line.

Among the targeted engineering strategies discussed in this review, glycosylation is the most critical. Obtaining homogenous glycosylation patterns is practically impossible without genetic manipulation or chemical treatment of the cell

line [57]. Two HEK293 cell lines with improved glycosylation profiles are currently in use; the commercially available HEK293SG (HEK293SGnTI$^-$ (ATCC® CRL-3022™), in which the N-acetyl-glucosaminyltransferase I (GnTI) gene has been knocked out and lacks complex N-glycans, and its derivative cell line HEK293SGGD (293SGlycoDelete (RRID:CVCL_6E38), which expresses a heterologous Golgi endoglycosidase for secretion of de-glycosylated glycoproteins. In addition, treatment of cells with small molecule inhibitors such as kifunensine, that targets α-mannosidase I and swainsonine, that blocks α-mannosidase II, results in relatively simple and chemically uniform glycans [58].

The majority of the currently approved therapeutics are being produced in derivative cell lines, underscoring the relevance of their unique properties; the recombinant FVIIIFc and recombinant FIXFc (ALPROLIX®, 2014; ELOCTATE®, respectively) in HEK293-H, the Human-cl rhFVIII (NUWIQ®) produced in HEK293F, the Glucagon-1-like peptide (GLP-1) Fc fusion protein (dulaglutide, TRULICITY®) produced in HEK293 EBNA, while the currently withdrawn drotrecogin alfa (XIGRIS®) was produced in the parental HEK293 cell line [13].

On the VLP and viral vector production front, parental HEK293 and derivatives such as HEK 293T and HEK293FT are the preferred hosts, the latter two due to the presence of the SV40 large T-antigen in their genomes that increases their viral production efficiency. Virus-like particle (VLP for vaccine generation) produced in HEK293 cell line include Rabies virus G protein VLP, Influenza virus A/Puerto Rico/8/34 (H1N1) VLP, Hepatitis B virus small surface antigen (HBaAgS) VLP, Human scavenger receptor class B, member 2 (SCARB2) VLP; Lentiviral vectors for gene therapy: Kymriah™ (Lymphoblastic leukemia) and ZyntegloT™ (Beta-thalassemia); AAV gene therapy vectors: Glybera™ (Lipoprotein lipase deficiency), Luxturna™ (Leber congenital amaurosis), and Zolgensma™ (Spinal muscular atrophy type 1); retroviral gene therapy vectors: Rexin-G™ (Pancreatic cancer), Strimvelis™ (Adenosine deaminase—immunodeficiency), Zalmoxis™ (Leukemia) [59].The choice of HEK cell line for producing viral vectors is usually more straightforward compared to the production of recombinant proteins. For most recombinant proteins produced in the HEK293 cell line and its derivatives, the choice of cell line is assessed on a case-by-case basis, and it is not uncommon for researchers to test different cell lines for productivity before deciding. Notwithstanding, the HEK293H and HEK293F cell lines with their superior transfectivity, higher productivity, and faster growth to high densities in chemically defined media are currently the choice for large scale recombinant protein production.

The HEK 293 cell line, along with a few other human cell lines, currently serves as a valuable niche for the production of biotherapeutics that require human PTMs. It is also the cell line of choice for the production of recombinant gene therapy vectors. Whether or not this cell line will become as widely used as the CHO cell line in biotherapeutic production will depend on the continued technological achievements and research investments in further optimizing this cell line.

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