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The Scattered Twelve Tribes of HEK293

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Their ease of growth and transfection makes HEK293 cells a common cell culture in academic research. In addition, high transfection efficiency of HEK293 cells enable production of exogenous proteins or viruses for pharmaceutical and biomedical research purposes. Recently, HEK293 cells has gained attention due to it is versatility for transfection experiments, particularly the propagation of adenoviral-based and retroviral-based vectors during CART-T bioprocess. Since traceability is critical to pharmaceutical manufacturing process, we provide a mini review to clarify the historical development and intent use of different variants of HEK293 cells. This review should provide a key reference for the HEK293 variants' historical and developmental background.

Keywords: HEK293, CAR-T, 293 Variants.

Their ease of growth and transfection makes HEK293 cells a common cell culture in academic research. In addition, high transfection efficiency of HEK293 cells enable production of exogenous proteins or viruses for pharmaceutical and biomedical research purposes [1–3]. For example, HEK293 cells are frequently used to express potential biological drugs and/or therapeutic target during the discovery stage [1,4,5]. Recently, HEK293 cells has gained attention due to it is versatility for transfection experiments, particularly the propagation of adenoviral-based and retroviral-based vectors during CART-T bioprocess [6–9].

A human embryonic kidney cell line was derived from the kidney of a human embryo in Alex van der Eb's laboratory in Netherlands. [10] After several months of cultivation, the fast-growing variant was established as HEK293 cells. Later, the microarray study demonstrate that HEK293 cells express a multitude of neuron-specific genes, indicating that 293 cells were originated from an immature neuronal cell in the embryonic kidney [10]. From there, many variants of HEK293 cells were developed for specific purposes [11]. Here we review these "tribes" of HEK293 cells that are" scattered" around the world to fulfill their maker's purpose in cell culture studies.

The Scattered Twelve Tribes of HEK293

1. HEK293 - The HEK293 line is the original line established from a primary embryonic human kidney and transformed with sheared human adenovirus type 5 DNA. The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein [2].
2. HEK293S – HEK293S cell is the original HEK293 line in suspension of modified minimal Eagle's medium. Full adaptation took about 7 months, and the first passages were so difficult that the few cells that grew through are likely to have been almost clonal. The fully adapted cell line is known as 293S [11]

3. HEK293T - HEK293T is a HEK293 variant that expresses a temperature-sensitive allele of the SV40 T antigen . This enables the amplification of vectors containing the SV40 ori and thus considerably increases the protein expression levels during transient transfection. SV40 T forms a complex with and inhibits p53, possibly further compromising genome integrity [10,12].

4. HEK293F – HEK293F is a variant of HEK293 cells. HEK293F cells were cloned from the HEK293 cell line and adapted to commercial medium.

5. HEK293FT - HEK293FT is a fast growing variant of HEK293T. HEK293FT cells were cloned from the HEK293T cell line and adapted to commercial media [13]. HEK293FT is designed for lentiviral production. Similar to HEK293T, the 293FT cells stably express the SV40 large T antigen from the pCMVSPORT6TAg.neo plasmid. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter and is high-level and constitutive [13].

6. HEK293FTM - HEK293FTM cell is derived from 293 cells by stable transfection of an FRT-site containing plasmid and of a TetR expression plasmid. The FRT site can be 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. The 293FTM cells were designed for protein-protein interaction studies [11].

7. HEK293SG - HEK293SG was derived from HEK293S by ethylmethanesulfonate (EMS) induced mutation. A Ricin toxin-resistant clone was then selected to become HEK293SG. The line lacked N-acetylglucosaminyltransferase I activity (encoded by the *MGAT1* gene) and accordingly predominantly modifies glycoproteins with the $Man_5GlcNAc_2$ N-glycan. HEK293SG is used for the production of homogenously N-glycosylated proteins [11].

8. HEK293SGGD -HEK293SGGlycoDelete cell line (293SGGD) derives from 293SG through transfection with an expression plasmid for a Golgi-targeted form of endoT, an endoglycosidase from the fungus Trichoderma reesei. HEK293SGGD are mainly used for glycosylation study [11].

9. HEK293H – HEK293H were cloned from HEK293 cells by limiting dilution to select a clone with good adherence during plaque assays.

10. HEK293E - HEK293E cells are derived from the HEK293 cell line and used for propagation of plasmids and expression of recombinant proteins in mammalian cells. The cell line expresses the EBNA-1 protein for episomal replication of oriP-harboring plasmids [14,15].

11. HEK293MSR - HEK293MSR cell line is genetically engineered from HEK293 expresses the human macrophage scavenger receptor and strongly adheres to standard tissue culture plates for dependable results [16].

12. HEK293A - HEK293A cell is a subclone of the HEK293 cells with a relatively flat morphology. It facilitates the initial production, amplification and titering of replication-incompetent adenovirus. The cell line contains a stably integrated copy of the E1 gene that supplies the E1 proteins (E1a and E1b) required to generate recombinant adenovirus [17,18].

Concluding Remarks

HEK293 and its variants have been the most frequently used cells after HeLa in cell biology studies and after CHO in biotechnology [11]. Despite the widespread and historically long term productive exploitation in cell biology, biotechnology, and cancer research, the designed purposes of these 293 variants were are frequently misused [10,13]. Therefore, we review the HEK293 and its variants in order to produce a comprehensive guild line for the intended purpose of these variants in cell culture studies.

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