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Induced pluripotent stem cells: A new addition to the virologists armamentarium

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A B S T R A C T

A significant amount of our understanding of the molecular events occurring during viral replication has originated from studies utilizing cell lines. These cell lines are normally obtained by the culture of samples from spontaneously occurring tumors or are derived by genetic manipulation of primary cells. The genetic events inducing immortalization and/or transformation to allow continual passage in culture can have profound effects resulting in a marked loss of cell type fidelity. The development of induced pluripotent stem cells (iPSCs) has revolutionized the field of developmental biology and is ushering in an era of personalized medicine for a wide range of inherited genetic diseases. Previously, development of iPSCs required dedicated facilities as well as highly detailed technical knowledge. The pace of development in this field however has been so rapid, that iPSCs are moving into an era of "off the shelf" use, whereby the use and manipulation of these cells is well within the ability of the majority of laboratories with standard tissue culture facilities. The introduction of iPSCs to studies in the field of virology is still in its infancy, and so far has been largely confined to viruses that are difficult to propagate in other experimental systems, but it is likely that this technology will become a standard methodology in the virologists armamentarium.

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1. Introduction

Currently a significant amount of virology research is carried out using immortalized and/or transformed cell lines, as opposed to primary cells. This is primarily because human or animal primary cells have a limited replicative potential in culture. As first described by Hayflick, human and animal cells will undergo a maximum of 50 ± 10 population doublings before the cells become senescent and eventually die (Hayflick, 1979). Practically then, primary cells do not offer a stable, uniform and continuous supply of cells needed for many virological investigations. Thus, while primary cells can offer a valid model system, their limited replicative potential dramatically limits their usefulness, and inter-donor variation can be a significant source of experimental complication. Additionally, primary cells are not commonly available for the majority of cell types.

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Thus, only in certain cases, such as monocytes (Marinho et al., 2014) or monocyte derived dendritic cells (Olagnier et al., 2014) for dengue virus research, do primary cells offer a viable working alternative and hence the common use of immortalized and/or transformed cell lines which are capable of unlimited proliferation.

2. Brief history of iPSCs

The first human cell line developed was HeLa, which was established from a carcinoma of the uterine cervix in 1952 by the growth of cells from the tumour in supplemented media (Scherer et al., 1953). Since then many hundreds of cell lines have been developed, either through methodologies similar to that employed for HeLa, or through genetic manipulation of primary cells such as the over-expression of the simian virus (SV-40) large T-antigen in primary mouse embryo fibroblasts which was used to generate the HEK293T cell line (Zhu et al., 1991). These cells have the ability to grow continuously in cell culture and they provide an easy and useful platform to investigate the cellular mechanisms of infectious agents such as viruses. However, as a consequence of their transformation or immortalization, these cells express proteins that are not found in the *bona fide* cell type, and may additionally not express proteins found in the *bona fide* cell type. Even where a cell line has been shown to possess a particular characteristic, that characteristic can be lost after extensive passage (Yu et al., 1997). In addition, much research has shown that 20% or more of all cell lines are either mis-identified or cross-contaminated (as reviewed in (Cabrera et al., 2006; Hughes et al., 2007)).

Misidentification and cross contamination of cell lines can occur for a number of reasons. The American Type Culture Collection Standards Development Organization Workgroup ASN-0002 identified simple mislabeling of culture vessels and outgrowth of a contaminating cell type as primary reasons for cell line errors (American Type Culture Collection Standards Development Organization Workgroup, 2010). Misidentified cell lines can have significant implications. For example the ECV304 cell line often used in dengue virus research as a model for endothelial cells (Bonner and O'Sullivan, 1998; Bosch et al., 2002; Liew and Chow, 2004; Liew and Chow, 2006; Yang et al., 2013) has been known since 1999 to be the T24 bladder carcinoma cell line (Dirks et al., 1999). Similarly, Chang liver cells previously used in studies investigating dengue virus infection of liver cells (Lin et al., 2002; Lin et al., 2000) are in fact are Hela cells (American Type Culture Collection Standards Development Organization Workgroup, 2010).

A solution to the problems with both primary cells and transformed cell lines lies in the application of induced pluripotent stem cells (iPSCs), which are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state. The first type of stem cell isolated was derived from a teratocarcinoma, and this type of stem cell is usually called an embryonal carcinoma cell (Kleinsmith and Pierce, 1964). Further studies subsequently led to the isolation of embryonic stem cells which were originally isolated from mouse (Evans and Kaufman, 1981; Martin, 1981), and subsequently from humans (Thomson et al., 1998). These cells are pluripotent and can differentiate into the three primary germ layers (ectoderm, endoderm and mesoderm) that generate all of the more than 200 adult human cell types. Embryonic stem cells are different from normal adult stem cells which are multipotent and are only capable of being differentiated into a few cell types. Embryonic stem (ES) cells can be maintained indefinitely in culture, and can be induced to differentiate into specific lineages (Rathjen et al., 1998). However, significant ethical concerns with human ES cells which must be harvested from pre-implantation human embryos have largely precluded their wider application. However, in 2006 Kazutoshi Takahashi and Shinya Yamanaka showed that the introduction of 4 specific genes (Oct4, Sox2, Klf4 and cMyc; also known as the Yamanaka factors or OSKM) that encoded transcription factors into an adult cell could convert the normal adult cells to pluripotent stem cells (Takahashi and Yamanaka, 2006), the so called induced pluripotent stem cells (iPSCs). Human iPSCs were first generated in 2007 (Takahashi et al., 2007) from human fibroblasts, and this cell type is still the preferred starting cell type although other types of cell have been used to generate human iPSCs (Montserrat et al., 2012).

3. OSKM: the Yamanaka factors

The Yamanaka factors (Oct4, Sox2, Klf4 and cMyc (OSKM)) were identified through an elegant screen of 24 candidate reprogramming factors (Takahashi and Yamanaka, 2006). While other combinations of reprogramming factors such as Oct4, Sox2, Nanog and Lin28 have been shown to successfully reprogram normal adult cells to iPSCs (Yu et al., 2009), the OSKM factors are still widely used today. Oct4 (octamer-binding transcription factor 4; also known as Oct3/4 and POU5F1, POU domain, class 5, transcription factor 1) is a member of the POU family of proteins named after a common protein DNA binding domain originally identified in Pit-1, Oct-1 and Oct-2 and the nematode factor Unc-86 (reviewed in (Verrijzer and Van der Vliet, 1993)). However, other POU family members cannot functionally replace Oct4 in generating iPSCs and Oct4 is essential for generating pluripotency (Nichols et al., 1998).

Sox2 also known as SRY (sex determining region Y)-box 2 is a member of the Sox family of transcription factors which have diverse roles in sex determination, chondrogenesis, hematopoiesis, neural crest development and neurogenesis (reviewed in (Sarkar and Hochedlinger, 2013)). The Sox family members are characterized by a conserved high mobility group (HMG) DNA-binding domain of approximately 80 amino acids and the Sox family members are divided into subgroups based on amino acid similarity of the HMG domain (Wright et al., 1993). Sox2 normally functions to maintain ES self-renewal (Ura et al., 2011) and to mediate ectodermal and endodermal tissue formation during fetal development (Arnold et al., 2011).

Klf4 (Kruppel-like factor 4 also known as EZF (epithelial zinc finger protein) or GKLF (gut-enriched kruppel-like factor)) is a member of the family of Kruppel-like factors which consists of highly conserved zinc finger DNA-binding transcription factors (reviewed in (Swamynathan, 2010)). Klf4 has essential functions in cell differentiation and proliferation and can function as a tumour suppressor protein or promote oncogenesis depending upon cellular context (Rowland et al., 2005). While Klf4 is generally required to induce iPSCs with the other Yamanaka factors, it is dispensable in cells which endogenously express Klf4 (Ho et al., 2010).

cMyc, which belongs to the Myc family of transcription factors (reviewed in (Eilers, 1999)) is probably the best known of the Yamanaka factors. cMyc controls the expression of hundreds of genes, and dysregulation of cMyc expression is a common occurrence in oncogenesis (Ott, 2014). The requirement for cMyc as one of the Yamanaka factors has been questioned (Nakagawa et al., 2008; Wernig et al., 2008), but studies suggest that better quality iPSCs result from including cMyc as one of the reprogramming factors (Araki et al., 2011).

4. Application of iPSCs to virology

Human iPSCs have great potential for direct application to human disease, by generating patient specific cells that can be used to correct or repair damaged cell lineages in patients (Shtrichman et al., 2013). However, some problems remain, particularly in the method of induction of pluripotency, which often is achieved