Cell Biology International ISSN 1065-6995
doi: 10.1002/cbin.10137

MINI-REVIEW

Immortality of cell lines: challenges and advantages of establishment

Muhammad Irfan Maqsood[1], Maryam M. Matin[1,2]*, Ahmad Reza Bahrami[1,2] and Mohammad M. Ghasroldasht[1]

1 Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2 Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Cellular immortality happens upon impairment of cell-cycle checkpoint pathways (p53/p16/pRb), reactivation or up-regulation of telomerase enzyme, or upregulation of some oncogenes or oncoproteins leading to a higher rate of cell division.There are also some other factors and mechanisms involved in immortalisation, which need to be discovered. Immortalisation of cells derived from different sources and establishment of immortal cell lines has proven useful in understanding the molecular pathways governing cell developmental cascades in eukaryotic, especially human, cells. After the breakthrough of achieving the immortal cells and understanding their critical importance in the field of molecular biology, intense efforts have been dedicated to establish cell lines useful for elucidating the functions of telomerase, developmental lineage of progenitors, self-renewal potency, cellular transformation, differentiation patterns and some bioprocesses, like odontogenesis. Meanwhile, discovering the exact mechanisms of immortality, a major challenge for science yet, is believed to open new gateways toward understanding and treatment of cancer in the long term. This review summarises the methods involved in establishing immortality, its advantages and the challenges still being faced in this field.

Keywords: cell cycle pathways; cell lines; immortalisation; pluripotency; senescence; telomerase

Introduction

Immortality is established when a cell loses its cell cycle checkpoint pathways. The overriding of natural cellular senescence takes place when inactivation of p53/p16/pRb occurs during immortalisation protocols (Shay et al., 1991). The mechanism controlling cellular senescence and immortalisation was described as a two-stage mechanism (terms are explained in Table 1) according to which telomerase activity is a key factor in the establishment of immortality (Wright and Shay, 1992). Strahl and Blackburn (1996) discussed high activity of telomerase in cellular malignancy and proposed its inhibition as a method for treatment of cancer. Shortly after this proposal, Marusic et al. (1997) studied this activity in the human cancer cells carrying a mutant telomerase gene (HT-cell lines). Extending the work of Morales et al. (1999), Steinert et al. (2000) studied the immortality of cell lines established after introducing the telomerase and its function in the elongation of telomeres and excision of the exogenous genes with their role in M1 and M2 stages (Figure 1).

How to achieve immortality?

Immortality of cell lines could be achieved by different approaches, including ectopic expression of telomerase or telemorase reverse transcriptase (TERT), by mutating the *p53* and *pRb* genes, or introducing the oncogenes, as described in Figure 1. Viral vectors may be used for all the mentioned approaches, that is introduction of TERT and oncogenes or mutating the *p53*/*pRb*, as will be explained in the following sections.

Immortality establishment by telomerase or TERT

Immortality has been achieved by introducing telomerase as well as TERT into the cells (Klingelhutz et al., 1994; Tsai et al., 2010). The elongation of telomeres increases the stability of chromosomes, making the cells immortal (Morales et al., 1999). Chang et al. (2005) managed to overexpress the hTERT in endothelial cells in order to immortalise them. In other efforts fibroblast-like cells,

*Corresponding author: e-mail: matin@um.ac.ir

Table 1 Definitions of terms related to cellular immortality.

Term	Explanation
Two-stage mechanism	A proposal according to which two separate mechanisms, the mortality stage one (Ml) and two (M2), regulate cellular senescence and immortalisation (Wright and Shay, 1992)—Figure 1
Telomere and telomerase	Telomeres are the specific regions at the end of chromosomes containing highly repetitive DNA. Telomerases are the enzymes that stabilise the chromosomal ends by adding telomeric repeats of DNA
TERT	Telomerase reverse transcriptase is the catalytic subunit of telomerase which helps in the elongation of the telomeres by adding $(TTAGGG)_n$ repeats in all vertebrates
p53	p53 is a protein known as cell regulator encoded by the *tp53* gene, commonly known as the Master Watchman of genome, responsible for control of cell growth
p16	p16 is a tumour suppressor protein responsible for cell cycle regulation encoded by *Cdkn2a* gene. Abnormality in its function can cause a variety of cancers
pRb	Protein of retinoblastoma gene (pRb) is the inhibitor of cell cycle
Protein kinase-Cι (PKCι)	A downstream mediator in the phosphoinositide-3-kinase (PI-3-kinase) pathway
Telomere position effect (TPE)	TPE is a phenomenon known for silencing of genes positioned near the telomeres. In eukaryotes, it was considered as a silencing mechanism along with suppressors of position effect variegation [Su(var)s] and Polycomb group proteins (PcG)

named HEF1, were immortalised by infection with a retroviral vector expressing hTERT (Xu et al., 2004), and ectopic expression of TERT in human mesenchymal stem cells (hMSCs) also resulted in increasing their stem-like properties (Tsai et al., 2010).

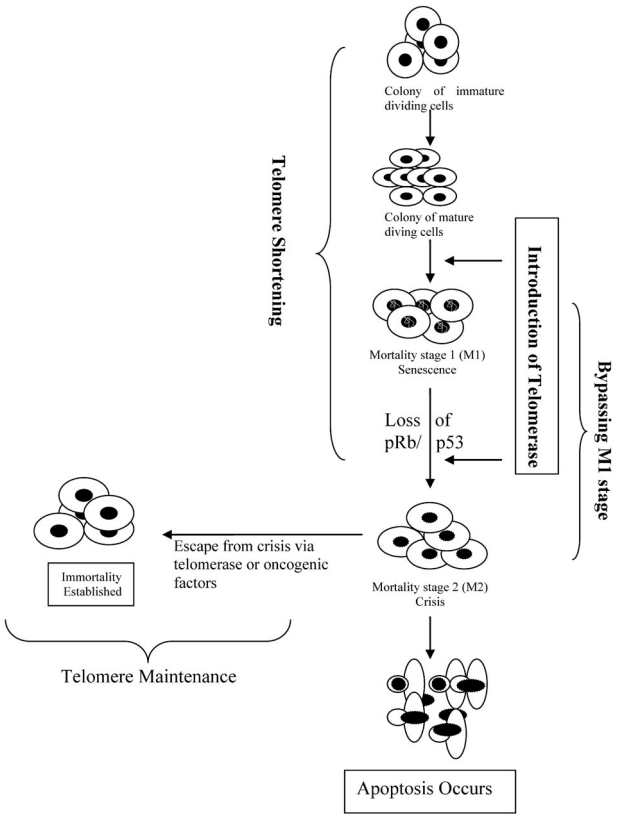

Figure 1 Immortalisation of human cells with ectopic expression of the hTERT.

Immortality establishment by mutating cell cycle checkpoints (p53/pRb)

Another way of achieving immortality is inactivation of the p53 and pRb (controllers of cell cycle) by introducing the E6 and E7 (human papillomavirus oncogenes) or E1A plus E1B (adenoviral oncogenes) proteins, or production of mutant versions by introducing simian virus 40 (SV40) (Shay et al., 1991). H-ras or K-rasoncoproteins from SV40 were also used to bypass or inactivate the p53/pRb checkpoints resulting in the immortalised transformation of bronchial epithelial cells (Lundberg et al., 2002). p53 and pRb are jointly considered as crucial factors for maintaining the cell cycle (Shay et al., 1991).

Immortality establishment by oncogenes and oncoproteins

Oncogenes or viral vectors encoding oncoproteins can also transform a cell into an immortalised state by silencing the cell cycle checkpoint pathways and cell cycle regulators. Human papillomavirus (HPV) and SV40 are widely used as vectors for such kind of transformations (Pereira-Smith and Smith, 1988). For example, anogenital epithelial cells were transformed using HPV (Klingelhutz et al., 1994). Introduction of *v-Myc* gene or a mutant form of *c-Myc* in human neural stem cells (hNSCs) also resulted in immortality of these cells (De Filippis et al., 2007, 2008). SV40 early genes were used in order to immortalise early embryonic cells (Kellermann and Kelly, 1986). Pereira-Smith and Smith (1988) also used SV40 to immortalise the lines of somatic cells. Controlled expression of genes like ribosomal protein P1 (RPLP1), cold-inducible RNA-binding protein (CIRP) and S-adenosylhomocysteine hydrolase (SAHH) have also been observed in immortalised human cancer cells (Artero-Castro

et al., 2009a,b; Lleonart et al., 2009). Oh et al. (2003) immortalised human B lymphocytes using Epstein–Barr virus. Immortality of human cervical and foreskin epithelial cells by the human papillomavirus type 16 or 18 (E6 and E7 open reading frames) was also achieved (Klingelhutz et al., 1994). Human embryonic kidney cells were also immortalised after the introduction of simian virus 40 and adenovirus (Counter et al., 1992). Transformed mammalian cell lines having different characteristics were established by introducing a temperature sensitive SV40 T-antigen (Chou, 1989).

Challenges in immortality

Transformation of cells to establish immortality is still a challenge for scientific communities. Many attempts have been made to induce immortality artificially, but reprogramming of all induced cells has shown some abnormal characteristics like induction of tumours. There are many hurdles in transforming a cell to become immortal. Some of the difficulties are summarised in Table 2.

Advantages of immortality

Immortality of cell lines is a rate-determining step in carcinogenesis that helps us to determine the continuous evolution and malignancy of cancers (Shay and Wright, 2005; Wu et al., 2003). Immortalisation helps to study the genes and factors involved in tumourigenesis (Wang et al., 2006). Relationships between telomerase expression and immortality have suggested various approaches in cancer therapy (Counter et al., 1992; Marusic et al., 1997). Oh et al. (2003) observed the properties of transformed B lymphocytes after immortalising the human B lymphocytes using Epstein–Barr virus. Much about cellular proliferation, its mechanism and proliferative capacity of cells has also been studied (Klingelhutz et al., 1994; De Filippis et al., 2008). The roles of temperature and environment on cellular proliferation have been studied by achieving immortalised cell lines (Chou, 1989; Jat and Sharp, 1989).

Hatano et al. (1991) tried to understand the cellular lineage and the mechanisms involved in differentiation. Behaviour of differentiated somatic cells and the differentiation pattern of cell lines (stem cell lines) can be studied after immortalising the cells (Sarin et al., 2005). Altered differentiative phenotypes after the establishment of immortality in cell lines suggest the relationship between immortality and phenotypes (Kohno et al., 2011). Differentiation of embryonic stem cells under different culture conditions can be studied by immortalising the neuronal cell lines, for example the differentiation of neurons from ESCs (Kornyei et al., 2005). The effects of TERT on gene regulation were studied and it was inferred that its effects were irrelevant to its catalytic enzyme action at telomeric ends (Tennen et al., 2011). The generation of immortal cell lines has proven useful to understand the molecular pathways governing mammalian cells (Bachoo et al., 2002). Cells were immortalised to study different processes like odontogenesis (Tsubakimoto et al., 2005). Different p53-dependent and -independent senescence pathways have been determined and considered as a tool in cancer repression (Ulanet and Hanahan, 2010; Basu et al., 2011; Chan et al., 2011; Paget et al., 2012). New biomarkers and epigenetic relationships have also been discovered in this regard (Collado et al., 2007; Choi et al., 2008; Simboeck et al., 2011).

Immortality of induced pluripotent stem cells has created many questions. Some possible protective mechanisms discussing the natural history of certain common cancers of man were described to maintain the cancer-free immortality of cells (Cairns, 1975). Non-random asymmetric segregation in distributed stem cells (DSCs) and satellite cells discussing the preservation of immortal DNA strands in stem cells have been studied (Shinin et al., 2006; Huh and Sherley, 2011). The maintenance in stem cells in relation to immortality remains a controversial question. Many researchers have proved the existence of immortal DNA strand, but others have challenged it. The discussion is ongoing regarding the asymmetric division of DNA in stem cells and the maintenance of immortal DNA to minimise the mutation rate (Li, 2007). Some studies have been analysed by Dolgin (2009), describing the features of tumour and immortalised or reprogrammed stem cells. The loss of stemness properties of stem cells with aging was described by Rando (2006), who thought that a stem cell loses its ability to overcome damage of tissues with aging. This loss of ability has led to the question of whether stem cells are mortal or immortal.

Immortalised cell lines

Establishment of immortality has remained a debatable question in scientific communities for several decades. Many researchers have tried to establish immortalised cell lines to study vital biological and molecular processes. Several advantages and challenges of their efforts arose over time and solutions have been found for some of the encountered problems. Table 3 summarises a number of immortalised cell lines which were produced using different techniques to study the secrets of molecular biology.

Conclusion

Immortality is a potential way to determine many life processes like cellular proliferation and differentiation, preservation of potency, determination of cellular lineages, malignancies of cancers, odontogenesis and other molecular

Table 2 Challenges being faced while immortalising the cells.

Issue	Description	References
Phenotypic relationships	Immortality remains a challenge in medicine due to its undiscovered relationship with cellular phenotypes. When immortality is established, cells do not keep their exact phenotype. Studying this changed phenotypic behaviour may be a key to establishing immortality in its ideal form	Kohno et al. (2011)
Epigenetic changes	The immortality of cell lines is affected by epigenetic modifications during cellular differentiation. Simboeck et al. (2011) described the epigenetic contribution in the establishment of immortality when studying the alterations in chromatin architecture affecting phenotypic and the epigenetic changes. Further studies are required to find out what contributions are provided by such epigenetic changes	Simboeck et al. (2011)
Cellular crises	Crisis before the arrival of immortality has been discussed as a serious problem in its establishment. At this point, the proportion of proliferative and apoptotic cells is important. Many attempts have been made in this regard to transform cells free from crisis. Collado et al. (2007) reviewed Hayflick factors involved in cellular senescence in normal and stem cells. More recently, p16INK4a tumour suppressor protein was considered as the biomarker acting as a biological clock to control the cellular senescence. The role of 3′-untranslated region (UTR) along with AU-rich element (ARE) and its cognate RNA-binding protein, HuR, in senescence-associated C/EBPβ target genes was also discussed in this regard	Goldstein (1990), McCormick and Campisi (1991), Wei and Sedivy (1999), Macera-Bloch et al. (2002), Collado et al. (2007), Basu et al. (2011)
p53/pRbindependent pathways	Many immortalised cancer cells, without mutations in p53 and/or pRb have been observed. Short but stabilised telomeres in immortalised human embryonic kidney cells have also been observed. Ulanet and Hanahan (2010) recently identified the role of Arf tumour suppressor in tumourigenesis through p53-independent mechanisms. Another breakthrough comes with the role of PKCι that its overexpression was also involved in immortalisation of cells	Counter et al. (1992), Chan et al. (2011), Ulanet and Hanahan (2010), Paget et al. (2012)
Telomere shortening	Telomere size (shortening and lengthening) has been studied as an important factor in the activation of DNA damage and senescence signals, controlling the mortality or immortality of cell lines. Satyanarayana et al. (2004) studied the initiation of DNA damage responses and senescence signalling as the overstimulation of the Ras/Raf/MEK/mitogen-activated protein kinase (MAPK) pathway seizing the telomere length. Recently, activation of cellular senescence was determined by two important biomarkers, CycE and E2F. The relationship between telomere dysfunction/size and senescent status of a cell needs to be investigated	Collado et al. (2007), Satyanarayana et al. (2004), Wang et al. (2009), Herbig et al. (2006)
Telomere position effect	Telomere position effect (TPE) in human cells and its relationship with immortality remain unexplained and a few connections between TPE and senescence have so far been shown. Chromatin-modifying factors that control TPE in yeast have been extensively studied and among these the lifespan regulator and silencing protein Sir2 has a pivotal role in the lifespan of the cells, but more studies are required in human cells	Ofir et al. (1999), Tennen et al. (2011), Doheny et al. (2008)
Telomerase and gene regulation	The role of TERT in gene regulation was considered as a crucial step in the maintenance of immortality. Many immortalised cancer cells with shortened telomeres have been observed. Regulation of telomerase and cellular proliferation was reviewed by Nicholls et al. (2011) for their involvement in cancer cell immortalisation. Epigenetic regulation of telomerase and the role of non-coding RNAs in its regulation have also been reviewed	Choi et al. (2008), Smith and Yeh (1992), Nicholls et al. (2011), Koziel et al. (2011)
Immortality and tumour	Immortalisation of cells in vitro is mostly associated with tumourigenesis, so it is still a challenge to achieve immortality free from tumourigenesis. Immortality established by the viral oncogenic expression, frequently results in senescence of the cells in spite of continued viral oncogene expression	Wang et al. (2006, 2009)

pathways in cells. It is beneficial for therapeutic purposes to achieve better results in the field of regenerative medicine and in fighting against some incurable diseases. The rapid breakthrough in embryonic stem cell research and its application is based on the immortality of cells. It could also be used to face and solve the challenges in the health sector. This review summarises the importance of immortality of cell lines and their potential applications, which can

Table 3 Immortalised cell lines.

Cell line	Immortalisation method	Purpose of study	Inferred result	Refs.
Fibroblast-like cells (HEF1)	Infection with a retroviral vector expressing hTERT	Change in the replicative lifespan of HEF1 (derived from hESCs)	Ectopic expression of TERT is responsible to restore the telomerase functionality and replicative lifespan may be increased	Xu et al. (2004)
Human neural stem cell lines	Using v-Myc gene	Proliferative capacity	Enhanced proliferative capacity	De Filippis et al. (2007)
Human B lymphocyte cell lines	Introduction of Epstein–Barr virus	Transformation effect of B lymphocytes	A specific strategy was developed to establish the immortalisation using Epstein–Barr virus	Oh et al. (2003)
Human cervical and foreskin epithelial cell lines	Introduction of human papillomavirus (HPV) type 16 or 18 E6 and E7 open reading frames	Relationship of telomere size and establishment of immortality	Pause in telomere shortening is necessary to establish immortality and proliferation ability is restored when size of telomere is restored	Klingelhutz et al. (1994)
Anogenital epithelial cell lines	Introduction of HPV	The activity of telomeres elongation	Longer telomeres are advantageous for the proliferation of cells	Klingelhutz et al. (1994)
Neuronal cell lines	Introduction of tsSV40T	The capacity of differentiation	Cells retained their differentiation capacity even when the oncogene was inactivated	Eves et al. (1992), Whittemore and White (1993), White et al. (1994), Barber et al. (2000)
Bone marrow derived hMSCs	Introduction of HPV16 E6 and E7 proteins	The phenomenon of spontaneous differentiation	The strategy for neuronal differentiation was developed as EZH2 may be repressed or knocked down to activate intracellular Ca^{2+} signalling	Hung et al. (2002)
Yolk sac cell lines	Retroviral-mediated expression of the *HOX11* homeobox-containing gene	The early haematopoietic development	A transitional stage may occur when yolk sac-derived cell lines differentiate and they have a little endothelial-like characteristics	Yu et al. (2002)
Rodent fibroblast cell lines	Introduction of herpes virus saimiri (HVS)	The expression of K1 in rodent fibroblasts	Morphological changes and foci formation, characteristics indicative of cellular transformation	Lee et al. (1998)

help cell biologists reach their targets, that is, treatment of diseases and improving health factors. Efforts are ongoing to study the immortality of cell lines in depth and to establish immortalised cell lines of differentiated and undifferentiated cells. Many challenges are assigned to the scientific communities to develop theoretical as well as practical approaches of having immortalised cell lines free from carcinogenic factors, such as epigenetic-based establishment of immortality. Some approaches have been developed and applied to remove such carcinogenic factors (Dawson et al., 2012; Li et al., 2013).

Acknowledgement and funding

We thank Ferdowsi University of Mashhad for providing a Ph.D. scholarship to M.I.M. and their financial support to perform this work.

References

Artero-Castro A, Callejas FB, Castellvi J, Kondoh H, Carnero A, Fernandez-Marcos PJ, Serrano M, Ramon y, Cajal S, Lleonart ME (2009a) Cold-inducible RNA-binding protein bypasses replicative senescence in primary cells through extracellular signal-regulated kinase 1 and 2 activation. Mol Cell Biol 29: 1855–68.

Artero-Castro A, Kondoh H, Fernandez-Marcos PJ, Serrano M, Ramon y, Cajal S, Lleonart ME (2009b) Rplp1 bypasses replicative senescence and contributes to transformation. Exp Cell Res 315: 1372–83.

Bachoo RM, Maher EA, Ligon KL, Sharpless NE, Chan SS, You MJ, Tang Y, DeFrances J, Stover E, Weissleder R, Rowitch DH, Louis DN, DePinho RA (2002) Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer Cell 1: 269–77.

Barber RD, Jaworsky DE, Yau KW, Ronnett GV (2000) Isolation and in vitro differentiation of conditionally immortalized murine olfactory receptor neurons. J Neurosci 20: 3695–704.

Basu SK, Malik R, Huggins CJ, Lee S, Sebastian T, Sakchaisri K, Quinones OA, Alvord WG, Johnson PF (2011) 3'UTR elements inhibit Ras-induced C/EBPβ post-translational activation and senescence in tumour cells. EMBO J 30: 3714–28.

Cairns J (1975) Mutation selection and the natural history of cancer. Nature 255: 197–200.

Chan CH, Gao Y, Moten A, Lin HK (2011) Novel ARF/p53-independent senescence pathways in cancer repression. J Mol Med (Berl) 89: 857–67.

Chang MW, Grillari J, Mayrhofer C, Fortschegger K, Allmaier G, Marzban G, Katinger H, Voglauer R (2005) Comparison of early passage, senescent and hTERT immortalized endothelial cells. Exp Cell Res 309: 121–36.

Choi J, Southworth LK, Sarin KY, Venteicher AS, Ma W, Chang W, Cheung P, Jun S, Artandi MK, Shah N, Kim SK, Artandi SE (2008) TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. PLoS Genet 4: e10.

Chou JY (1989) Differentiated mammalian cell lines immortalized by temperature-sensitive tumor viruses. Mol Endocrinol 3: 1511–4.

Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. Cell 130: 223–33.

Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 11: 1921–9.

Dawson MA, Kouzarides T, Huntly BJ (2012) Targeting epigenetic readers in cancer. N Engl J Med 367: 647–57.

De Filippis L, Lamorte G, Snyder EY, Malgaroli A, Vescovi AL (2007) A novel, immortal and multipotent human neural stem cell line generating functional neurons and oligodendrocytes. Stem Cells 25: 2312–21.

De Filippis L, Ferrari D, Rota Nodari B, Amati B, Snyder E, Vescovi AL (2008) Immortalization of human neural stem cells with the c-myc mutant T58A. PLoS ONE 3: e3310.

Doheny JG, Mottus R, Grigliatti TA (2008) Telomeric position effect—a third silencing mechanism in eukaryotes. PLoS ONE 3: e3864.

Dolgin E (2009) Immortality improves cell reprogramming. Nat News doi: 10.1038/news.2009.809.

Eves EM, Tucker MS, Roback JD, Downen M, Rosner MR, Wainer BH (1992) Immortal rat hippocampal cell lines exhibit neuronal and glial lineages and neurotrophin gene expression. Proc Natl Acad Sci USA 89: 4373–7.

Goldstein S (1990) Replicative senescence: the human fibroblast comes of age. Science 249: 1129–33.

Hatano M, Roberts CW, Minden M, Crist WM, Korsmeyer SJ (1991) Deregulation of a homeobox gene, HOX11, by the t (10;14) in T cell leukemia. Science 253: 79–82.

Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. Science 311: 1257.

Huh YH, Sherley JL (2011) Molecular cloaking of H2A.Z on mortal DNA chromosomes during nonrandom segregation. Stem Cells 29: 1620–7.

Hung SC, Chen NJ, Hsieh SL, Li H, Ma HL, Lo WH (2002) Isolation and characterization of size-sieved stem cells from human bone marrow. Stem Cells 20: 249–58.

Jat PS, Sharp PA (1989) Cell lines established by a temperature-sensitive simian virus 40 large-T-antigen gene are growth restricted at the nonpermissive temperature. Mol Cell Biol 9: 1672–81.

Kellermann O, Kelly F (1986) Immortalization of early embryonic cell derivatives after the transfer of the early region of simian virus 40 into F9 teratocarcinoma cells. Differentiation 32: 74–81.

Klingelhutz AJ, Barber SA, Smith PP, Dyer K, McDougall JK (1994) Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. Mol Cell Biol 14: 961–9.

Kohno S, Murata T, Koide N, Hikita K, Kaneda N (2011) Establishment and characterization of a noradrenergic adrenal chromaffin cell line, tsAM5NE, immortalized with the temperature-sensitive SV40 T-antigen. Cell Biol Int 35: 325–34.

Kornyei Z, Szlavik V, Szabo B, Gocza E, Czirok A, Madarasz E (2005) Humoral and contact interactions in astroglia/stem cell co-cultures in the course of glia-induced neurogenesis. Glia 49: 430–44.

Koziel JE, Fox MJ, Steding CE, Sprouse AA, Herbert BS (2011) Medical genetics and epigenetics of telomerase. J Cell Mol Med 15: 457–67.

Lee H, Veazey R, Williams K, Li M, Guo J, Neipel F, Fleckenstein B, Lackner A, Desrosiers RC, Jung JU (1998) Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herpesvirus. Nat Med 4: 435–40.

Li L (2007) Does immortal DNA strand exist in immortal stem cells? Cell Res 17: 834–5.

Li KK, Li F, Li QS, Yang K, Jin B (2013) DNA methylation as a target of epigenetic therapeutics in cancer. Anticancer Agents Med Chem 13: 242–7.

Lleonart ME, Artero-Castro A, Kondoh H (2009) Senescence induction; a possible cancer therapy. Mol Cancer 8: 3.

Lundberg AS, Randell SH, Stewart SA, Elenbaas B, Hartwell KA, Brooks MW, Fleming MD, Olsen JC, Miller SW, Weinberg RA, Hahn WC (2002) Immortalization and transformation of primary human airway epithelial cells by gene transfer. Oncogene 21: 4577–86.

Macera-Bloch L, Houghton J, Lenahan M, Jha KK, Ozer HL (2002) Termination of lifespan of SV40-transformed human fibroblasts in crisis is due to apoptosis. J Cell Physiol 190: 332–44.

Marusic L, Anton M, Tidy A, Wang P, Villeponteau B, Bacchetti S (1997) Reprogramming of telomerase by expression of mutant telomerase RNA template in human cells leads to altered telomeres that correlate with reduced cell viability. Mol Cell Biol 17: 6394–401.

McCormick A, Campisi J (1991) Cellular aging and senescence. Curr Opin Cell Biol 3: 230–4.

Morales CP, Holt SE, Ouellette M, Kaur KJ, Yan Y, Wilson KS, White MA, Wright WE, Shay JW (1999) Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 21: 115–8.

Nicholls C, Li H, Wang JQ, Liu JP (2011) Molecular regulation of telomerase activity in aging. Protein Cell 2: 726–38.

Ofir R, Wong AC, McDermid HE, Skorecki KL, Selig S (1999) Position effect of human telomeric repeats on replication timing. Proc Natl Acad Sci USA 96: 11434–9.

Oh HM, Oh JM, Choi SC, Kim SW, Han WC, Kim TH, Park DS, Jun CD (2003) An efficient method for the rapid establishment of Epstein–Barr virus immortalization of human B lymphocytes. Cell Prolif 36: 191–7.

Paget JA, Restall IJ, Daneshmand M, Mersereau JA, Simard MA, Parolin DA, Lavictoire SJ, Amin MS, Islam S, Lorimer IA (2012) Repression of cancer cell senescence by PKCiota. Oncogene 31: 3584–96.

Pereira-Smith OM, Smith JR (1988) Genetic analysis of indefinite division in human cells: identification of four complementation groups. Proc Natl Acad Sci USA 85: 6042–6.

Rando TA (2006) Stem cells, ageing and the quest for immortality. Nature 441: 1080–6.

Sarin KY, Cheung P, Gilison D, Lee E, Tennen RI, Wang E, Artandi MK, Oro AE, Artandi SE (2005) Conditional telomerase induction causes proliferation of hair follicle stem cells. Nature 436: 1048–52.

Satyanarayana A, Greenberg RA, Schaetzlein S, Buer J, Masutomi K, Hahn WC, Zimmermann S, Martens U, Manns MP, Rudolph KL (2004) Mitogen stimulation cooperates with telomere shortening to activate DNA damage responses and senescence signaling. Mol Cell Biol 24: 5459–74.

Shay JW, Wright WE (2005) Senescence and immortalization: role of telomeres and telomerase. Carcinogenesis 26: 867–74.

Shay JW, Wright WE, Werbin H (1991) Defining the molecular mechanisms of human cell immortalization. Biochim Biophys Acta 1072: 1–7.

Shinin V, Gayraud-Morel B, Gomes D, Tajbakhsh S (2006) Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells. Nat Cell Biol 8: 677–87.

Simboeck E, Ribeiro JD, Teichmann S, Di Croce L (2011) Epigenetics and senescence: learning from the INK4-ARF locus. Biochem Pharmacol 82: 1361–70.

Smith JK, Yeh G (1992) Telomere reduction in endometrial adenocarcinoma. Am J Obstet Gynecol 167: 1883–7.

Steinert S, Shay JW, Wright WE (2000) Transient expression of human telomerase extends the life span of normal human fibroblasts. Biochem Biophys Res Commun 273: 1095–8.

Strahl C, Blackburn EH (1996) Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. Mol Cell Biol 16: 53–65.

Tennen RI, Bua DJ, Wright WE, Chua KF (2011) SIRT6 is required for maintenance of telomere position effect in human cells. Nat Commun 2: 433.

Tsai CC, Chen CL, Liu HC, Lee YT, Wang HW, Hou LT, Hung SC (2010) Overexpression of hTERT increases stem-like properties and decreases spontaneous differentiation in human mesenchymal stem cell lines. J Biomed Sci 17: 64.

Tsubakimoto T, Saito M, Tsunoda A, Hattori S, Nishida E, Kousaka K, Toyoda M, Sato S, Noguchi T, Teramaka T (2005) Molecular mechanisms of periodontal development and its prospect of regenerative medicine for periodontium. Bull Kanagawa Dent Coll 33: 31–5.

Ulanet DB, Hanahan D (2010) Loss of p19(Arf) facilitates the angiogenic switch and tumor initiation in a multi-stage cancer model via p53-dependent and independent mechanisms. PLoS ONE 5: e12454.

Wang L, Dittmer DP, Tomlinson CC, Fakhari FD, Damania B (2006) Immortalization of primary endothelial cells by the K1 protein of Kaposi's sarcoma-associated herpesvirus. Cancer Res 66: 3658–66.

Wang C, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, von Zglinicki T (2009) DNA damage response and cellular senescence in tissues of aging mice. Aging Cell 8: 311–23.

Wei W, Sedivy JM (1999) Differentiation between senescence (M1) and crisis (M2) in human fibroblast cultures. Exp Cell Res 253: 519–22.

White LA, Eaton MJ, Castro MC, Klose KJ, Globus MY, Shaw G, Whittemore SR (1994) Distinct regulatory pathways control neurofilament expression and neurotransmitter synthesis in immortalized serotonergic neurons. J Neurosci 14: 6744–53.

Whittemore SR, White LA (1993) Target regulation of neuronal differentiation in a temperature-sensitive cell line derived from medullary raphe. Brain Res 615: 27–40.

Wright WE, Shay JW (1992) The two-stage mechanism controlling cellular senescence and immortalization. Exp Gerontol 27: 383–9.

Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, Luo S, Hong WK, Spitz MR (2003) Telomere dysfunction: a potential cancer predisposition factor. J Natl Cancer Inst 95: 1211–8.

Xu C, Jiang J, Sottile V, McWhir J, Lebkowski J, Carpenter MK (2004) Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth. Stem Cells 22: 972–80.

Yu WM, Hawley TS, Hawley RG, Qu CK (2002) Immortalization of yolk sac-derived precursor cells. Blood 100: 3828–31.

Received 18 December 2012; accepted 2 May 2013.
Final version published online 24 June 2013.