Immortalization Strategies

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What is Immortalization?

Cellular immortalization is the acquisition of a theoretical infinite life span[1,2], and it is a characteristic present in tumoral cells, as well as some established cell lines. Previous studies have shown that tumorigenesis *in vivo*[3] as well as viral-mediated transformation *in vitro*[4, 5] is a two-step process; the first step being converting the normal cells into immortalized cells, escaping senescence, and the second step consisting in transforming the immortalized cells into their neoplastic state. Different agents act on different steps of the process, although some genetic agents can have both immortalizing and transforming activity[6].

Therefore immortalization is an intermediate state between a normal and a malignant cell, with an infinite life span – cell strains that are capable of growing for 100 population doublings and can survive subsequent subcloning are considered to be immortal[1,7]. Between immortalized cell lines, a continuum of sub-states can exist, one cell line can remain differentiated and only differing with the normal line in their infinite life span, while other immortalized cell strains can have retain some differentiated functions but not all. This difference can be attributed to the particular cell line and immortalizing agent used.

The biological mechanisms behind this process and its complex interactions are still not well understood[1]; but to put it briefly, agents that can affect the expression or function of genes involved in the negative control of the cell cycle[1,2,7] or up-regulate positive elements, like the proteic catalytic subunit of the telomerase, hTERT have immortalizing potential.

There are several ways of inducing immortalization. Spontaneous mutations can give rise to some immortalized cell lines (e.g. 3T3 cell lines[8]), but it is a rare event that do not usually occur with most of the cell lines. Physical agents like radiation or chemicals can also induce immortalization[9], but because of its non-specific effect on the cell machinery, are undesirable to use. Viral genes or whole viruses are the preferred method for establishing immortalized cell lines; because of its immortalizing activity and efficiencies[1,7,10]. Several oncogenes and the hTERT gene have been also used to immortalize cells[6,7,11-14]. Lastly, the hybridoma technology[15] allows to fuse antibody-expressing B-cells with myeloma cells, giving a immortal hybrid cell strain.

Viral Genes

Viral genes are the most used agent to immortalize cells, due to their great efficiency and proven immortalizing activity. Viral genes interact with suppressors of the cell cycle (like CIP-1/WAF-1/p21, Rb, p53 and p16) and destabilize DNA surveillance machinery, increasing the life span of the cells and enhancing the occurrence of mutations that can effectively cause an escape from senescence[1]. The main inconvenience when using viral genes is that these can cause a little dedifferentiation[1,2,7,10].

SV40LTag

The most employed gene for immortalization is the **Large Antigen T gene from the simian virus SV40** (SV40LTag), it has been successfully used to immortalize cells from various lineages from several mammals[1,2,7,10,16]. There are several ways to introduce the gene into the cell, depending on the characteristics of the cell line (see Table 1).
Because of its weak but undesirable transforming activity, a temperature-sensitive mutant has been created (SV40tsLTag)[2,10], allowing to change between an immortalized partially dedifferentiated cell to a mortal but totally differentiated cell.
Also, a transgenic mice line has been established (Immortomouse)[17], that allows via γ-interferon to regulate the expression of the SV40tsLTag gene, and can be used to establish several immortalized cell lines.

Method	Advantages	Disadvantages	Target cells
Calcium phosphate precipitation	Cheap Robust	Toxic Low transfer efficiency	Large quantity of cells Not sensitive to calcium phosphate
Strontium phosphate precipitation	Cheap Robust Low toxicity	Low transfer efficiency	Large quantity of cells Sensitive to calcium phosphate
Liposomes	Low toxicity Good transfer efficiency	Expensive, requires optimization	Small quantity of cells Sensitive to calcium phosphate
Electroporation	Can be applied to cells in suspension	Low transfer efficiency Expensive	Large quantity of cells Grow in suspension
Microinjection	Very high transfer efficiency	Expensive and complex Safety considerations	Small quantity of cells Sensitive to calcium phosphate
Retroviral vectors	Very high transfer efficiency	Specialized facilities Nondividing cells infection is not possible	Small quantity of cells Dividing cells Sensitive to calcium phosphate
Adenoviral vectors	Very high transfer efficiency Nondividing cells infection	Specialized facilities No SV40tsLTag construct available	Small quantity of cells Nondividing cells Sensitive to calcium phosphate

Table 1. Different methods for introducing the SV40LTag gene in the cells. [10]

Other viral genes and viruses

Other viral genes or even whole viruses have been used to establish immortal cell lines, and although they show a weaker immortalizing activity than SV40LTag, the cells immortalized often retain their differentiated phenotype[1,7]. Among others:

- **Epstein-Barr Virus** (EBV) has infected and been extensively used to immortalize lymphoblastoid cells that produce antibodies[18,19].
- **Adenovirus E1a gene** has been used to immortalize rat baby kidney cells[20], embryo fibroblasts[21] and in conjunction with E1b, rat differentiated hepatocytes[22].
- **Human Papilloma Virus** (HPV) **E6 and E7 genes** have been used to immortalized a great number of cell types (several epithelia, endothelia, hepatocytes, melanocytes)[16].

Other Genes

Genes involved in the regulation of the cell cycle and cell aging can also induce immortalization when ectopically expressed; although some oncogenes have also transforming activity and the resultant strain usually express a dedifferentiated phenotype.

hTERT

Transfection with the **proteic catalytic subunit of the telomerase** (hTERT) can extend the life span of the cell line, a high fraction of the cells will become immortal but not transformed[11,12], and their phenotype remains differentiated. Keratinocytes[13] and myocytes[14] are among the lineages immortalized with this technique.

Oncogenes

Oncogenes such as ***myc***, ***ras*** and ***p53*** have been used to establish several cell lines, which conserved partially their phenotype[6,7].

Hybrid Cell Lines

Studies of somatic fusion between finite and immortal cell lines have shown that usually the immortalized phenotype is recessive, and that the senescence genes are dominant[23], although there are exceptions not limited to hybridomas[24].

Hybridomas

Hybridomas[15] are the result of the fusion of neoplastic B-cells with splenocytes from an immunized animal, creating an immortal hybrid cell line that produces monoclonal antibodies. Auxotrophic strains are used for selection in HAT medium.

Conclusions

When choosing which approach follow, it is necessary to study the characteristics and availability of the selected cell line, but with the knowledge exposed here, good rules of thumb would be:

- Review the literature.
- If there is a large supply of cells to try first with the cheapest and most extended method, transfection of the SV40LTag by calcium phosphate precipitation.
- If there is a small supply of cells, recombinant viral vectors with SV40LTag.
- If the differentiated expression could be compromised, use SV40tsLTag or hTERT.

Selected Bibliography

1. Freshney RI (2005). *Culture of Animal Cells: A Manual of Basic Technique*. 5th Ed. Wiley-Liss. Hoboken, 642p.
2. McLean JS (1993). *Improved techniques for immortalizing animal cells*, Trends in Biotechnology, Volume 11, Issue 6: 232-8.
7. MacDonald C (1990). *Development of New Cell Lines for Animal Cell Biotechnology*. Crit. Rev. Biotechnol. 10: 155–78.