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# A new sight of reprogramming efficiency pattern from different somatic cells in Chinese traditional medicine

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**Abstract**
The discovery of induced pluripotent stem cells (iPS) has been proven as a breakthrough since they have significant the advantages over embryonic stem cells in clinical application. Following the discovery of iPS cells, many sources and methods of producing them have emerged. Each method displays different reprogramming efficiency, a key element in both the clinical application of iPS technology and the research of iPS properties. Human iPS cells can be generated from different types of somatic cells, which could be divided into different viscera categories in the Traditional Chinese Medicine (TCM) theory. Interestingly, the reprogramming efficiency of different somatic cells shows a special relationship with the viscera categories to which those cells belong. This review investigates the reprogramming efficiency of human iPS cells from different somatic cells and demonstrates the connection between reprogramming efficiency and TCM viscera theory. Here, the comparison data led to a prediction on the reprogramming efficiency of the somatic cells without being reported. Hopefully, the findings may provide more useful information for iPS cells selection in clinical use and serve as the first step for the construction of a reprogramming efficiency data pool.

**Keywords:** Different somatic cells, cell types, OSKM, Yamanaka factors, iPS generation, reprogramming efficiency, Traditional Chinese Medicine (TCM) theory

## Introduction
Since the 1960s, scientists have explored the clinical application of stem cells, especially to healing tissue and organ damage [1]. However, ethical problems and immunological rejection have deterred scientists from fully applying stem cell technology to solve real-life medical problems [2]. The discovery of the iPS cell production in 2006 [3], proved to be the long-awaited breakthrough [4]. As known, the iPS cells are generated from somatic cells, showing the similar characters as that of embryonic stem cells [5]. Nowadays, the iPS cells have been differentiated into many types of cells, including hepatocytes [6], retinal ganglion cells [7], and neural progenitor cells [8]. These resulting cells are considered to have great potentials to repair many kinds of damage to the human body [9].
The creation of iPS cells that are almost morphologically and functionally identical to embryonic stem cells requires multiple steps. After extracting somatic cells from patients, scientists can use various gene delivery systems to transduce the cells. Then, they incubate these transduced cells in a specific culture environment [10]. After colonies emerge, scientists use characterizations such as alkaline phosphatase (AP) staining, karyotype, gene expression, and teratomas formation to identify the reprogrammed cells.Since the extraction of embryonic stem cells will cause the death of the embryo, iPS cells are easier to obtain, more abundant, and more ethical to acquire. Moreover, cells from the same host can integrate smoothly into the host system. Therefore, iPS cells are not likely to trigger immunological rejection. These two reasons give great potential to iPS cells for clinical application.

## Somatic Cell Types, Reprogramming Methods and Reprogramming Efficiency
After the discovery of iPS cell reprogramming using fibroblast in 2006 [11], scientists have attempted to induce many different types of somatic cells into iPS cells, including fibroblasts, stomach cells, pancreatic beta cells [12], adipose tissues, primordial germ cells [13] and others (Table 1) [14]. Excitingly, cells from urine could be generated into iPS cells as well: The urine cells showed higher reprogramming efficiency rates than that of blood cells

and skin cells, being an ideal cell source of human somatic cells [15]. Building on initial efforts, a non-integrating human induced pluripotent stem cell bank from urine-derived cells was set up, indicating a feeder-free, virus-free and serum-free condition with a easier somatic cell source bloomed up. This would be great helpful to obtain cells from patients, further therapeutic research and the future clinical use [16].

Meanwhile, many cell transduction methods are discovered to reprogram somatic cells, including methods that involve the use of lentivirus, retrovirus, adenovirus [17], plasmid [18], protein [19], cell culture condition [20], micro RNAs [21], or Sendai viruses [22]

**Table 1** [23]

Sources of Adult Somatic Cells for generation of iPSCs

| Sources | Authors |
|---|---|
| Fibroblasts | 17,20 |
| Stomach cells | 62 |
| Liver cells | 23,62 |
| Neural progenitor cells | 63,64 |
| Lymphocytes | 63 |
| B- cells | 65 |
| Keratinocytes | 22 |
| Human blood | 66,67 |
| Human cord blood | 68,69 |
| Human adipose tissues | 70 |

Although scientists have come up with various combinations of ways to reprogram somatic cells into iPS, the efficiency of reprogramming is one of the most-watched points, especially due to the clinical use or the construction of disease models [24]. Low efficiency imposes severe limitations on the biomedical utility of the reprogramming method [25]. By contrast, as known, high efficiency can save the time and increase the chances of experimental success, therefore lowering the financial cost of experimentation in the long-run. Reprogramming efficiency varies depending on the somatic cell type.[26] [27] In this review, the reprogramming efficiency rates of reported somatic cells are horizontally catalogued and compared. The fantastic connection is detected between reprogramming efficiency of different somatic cells and the categories the cells fell into in TCM theory. For example, the cells related to kidney categories such as renal tubular cells show generally higher reprogramming efficiency than that of the cells related to liver categories such as Hepatocytes. Meanwhile, the cells related to liver categories have higher reprogramming efficiency than that of the cells related to Defense-Qi categories such as fibroblasts. The retrovirus transduction with the OSKM transcription factors (Oct4, Sox2, Klf4, and c-Myc), as the most basic reprogramming method, is used to isolate the reprogramming experimental examples in this analysis with TCM theory.

**Reprogramming Efficiency Rates**

1. Yamanaka *et al.* first transduced human fibroblast into iPS cells with a reprograming efficiency rate of 0.01% to 0.022%[28].
2. Cai *et al.* reported the generation of iPS cells from the placental amniotic membrane with a 0.1% efficiency rate[29].
3. Using the same method, Cai *et al.* transduced the mesenchymal cells of the umbilical cord matrix into iPS cells with an efficiency rate of up to 0.4% [30].
4. Zhou reported the generation of human iPS cells from exfoliated renal tubular cells present in urine with an efficiency rate of up to 4% [31]. In the meantime, Cai and Zhou produced green fluorescent protein (GFP) as a control using parallel transduction with retroviruses.
5. Aasen *et al*. reported the reprogramming of juvenile human primary keratinocytes in 2008 with an efficiency rate close to 1% [32].
6. Liu *et al*. first reprogrammed human primary hepatocytes into pluripotency in 2010, observing an efficiency rate close to 0.1 to 0.2% [33].
7. Loh *et al*. produced iPS cells from CD34+ mobilized human peripheral blood cells, observing five to ten hES cell-like colonies from 50,000 CD34+ cells. presenting an reprogramming efficiency rate between 0.01% and 0.02% [34].
8. Lagarkova *et al*. generated iPS cells from human umbilical vein endothelial cells (HUVeC) in 2010 with a reprogramming efficiency rate of approximately 0.03% [35].
9. Miyoshi *et al*. generated iPS cells from oral mucosa with an efficiency rate of 0.022% [36].
10. Brown *et al*. derived iPS cells from human peripheral blood T lymphocytes with an efficiency rate of approximately 0.01%, similar to the published efficiency rates of fibroblasts and CD34+ cells [37].
11. Generation of iPS cells from human astrocytes has "a similar reprogrammingefficiency as keratinocytes, which was much higher when compared to fibroblasts." [38]
12. After double infection, the efficiency rate of dental pulp stem (DPS) cells can also reach 0.1%.[39]Multiple articles may collectively prove the efficiency of one type of cell, but only one of them is presented above.

The reprogramming efficiency rate for each kind of somatic cell is presented in Table 2.

**Table 2**

| Cell Category | | Somatic Cell Type Transduced by Retrovirus with OSKM Factors | Reprogramming Efficiency Rate |
|---|---|---|---|
| Kidney group | | Exfoliated renal tubular cells present in urine | Up to 4% |
| | | Keratinocytes (from hair) | ~1% |
| | | Human astrocytes | ~1% |
| Liver group | | Amniocytes from amnion | 0.1% |
| | | Hepatocytes (liver) | ~0.1%-0.2% |
| Defense-Qi (defense System) group | Group three | Umbilical vein endothelial cells (HUVEC) | ~0.03% |
| | | Oral mucosa fibroblast (OFs) | ~0.022% |
| | Group four | Peripheral blood T lymphocytes | 0.01% (similar to fibroblast and CD34+ efficiency rates) |
| | | Human fibroblasts[40] [41] [42] | 0.01%-0.022% |
| | | CD34+, mobilized human peripheral blood cells | 0.01%-0.02% |
| Cells at different stages | | Immature dental pulp stem cell (IDPSCs) [43] | Significantly higher efficiency than fibroblast |
| | | Umbilical cord blood's MSCs | Up to 0.4% |
| | | Dental pulp stem (DPS) cells | 0.1% |
| | | Stem cell from human exfoliated deciduous teeth (SHED) [44] | Significantly higher efficiency than fibroblasts |
| | | Hematopoietic progenitors[45] | Up to 28% |

**Regular Pattern and Relationship to TCM Viscera Theory**

Based on significant differences in reprogramming efficiency rates between different somatic cell groups under retrovirus infection system with OSKM factors, the cells are categorized into four groups.

The first group, tentatively called the kidney group, comprises of cells that have reprogramming efficiency rates equal to or higher than 1%. Hair keratinocytes, human astrocytes, and exfoliated renal tubular cells present in urine belong to the kidney group.

The second group, tentatively called the liver group, consists of cells that have efficiency rates equal to or higher than 0.1% while lower and 1%. It includes amniocytes from the inner layer of placenta and hepatocytes.

The third group is composed of cells that have efficiency rates higher than 0.01% and lower than 0.1%. It contains umbilical vein endothelial cells and oral mucosa fibroblasts.

The fourth group comprises of cells that have efficiency rates close to 0.01% and includes peripheral blood T lymphocytes, CD34+ mobilized human peripheral blood cells, and skin fibroblasts. The similarities between the efficiency rates of cells in the third and fourth groups lead me to categorize them together as the defense-Qi (defense system) group. Since the reprogramming efficiency of somatic cells (e.g. dental pulp stem cells) undergoing different cell differentiation stages cannot be taken into account, these cells are separately grouped together.

Based on the knowledge of the *Yellow Emperor's Canon of Medicine* (YECM) [46], one of the classics of TCM theory, an interesting phenomenon is shown: cells related to kidney seem to have higher reprogramming efficiency than those related to liver which have higher reprogramming efficiency than those related to Defense-Qi. The detailed analysis is presented in the following sections.

**The kidney group**

According to Traditional Chinese Medicine theory, the somatic cell types in the first group are closely related to kidney. Firstly, the renal tubular cells evidently relates to kidney. Secondly, as for Human Astrocytes, it belongs to brain and marrow which are close related to kidney according to the description in classic of Chinese Traditional Medicine(TCM), Yellow Emperor's Canon of Medicine(YECM). " the brain is the sea of marrow" and "all marrow is related to the brain" while "the kidney produces bone marrow." Therefore, from the perspective of TCM, kidney is closely related with brain and marrow from which atrocities come. In terms of hair Keratinocytes, YECM states clearly its relation with kidney that "The kidney manages closure and is the root of storage and the house of Jing (essence). The kidney demonstrates its Hua (splendor) on the Fa (hair), nourishing the bones." Therefore, after the explanation from the YECM, all cells in the highest reprograming efficiency group closely connect with kidney.

**The liver group**

Somatic cells in this group are mainly related with liver, resulting in lower reprogramming efficiency. Hepatocytes self-evidently belong to liver group. In terms of Amniocyte, it comes from amnion, which is called "the cloth of embryo" in Chinese traditional classics. According to *Ken Tang Yi Lun*, "the cloth of embryo (amnion), taste salty, smell moderate, enters the Liver channel." In addition, the current scientific definition of amnion said that "as the most inner layer of placenta, amnion has similar structure to human conjunctiva, containing Ocular surface epithelial cell, including conjunctiva cells and other needed materials for the growth of corneal epithelial cells [47]." From this definition, we can learn that amnion has close relationship to "eye" in traditional Chinese Medicine Theory. According to YECM, "Qibo answered, 'the east produces wind, the wind promotes (the growth) of trees, the trees produces sour (taste), the sour (taste) nourishes the liver, (the blood stored in) the liver nourishes the sinews, the sinews nourishes the heart and the liver controls the eyes.'" It means that kidney is closed to eye which is firmly connected to amnion. Based on this, amnion is closest related to liver.

**The Defense-Qi group (Surface-Qi group)**

Comparing with two previous groups, somatic cell types in the third and the forth group have very low reprogramming efficiencies which are all lower than 0.03%. At the meantime, they are closely related to human immune

system. According to YECM, "Weiqi (Defensive-Qi) functions to warm the muscles, moisten the skin, fill in the Couli (muscular interstices) and control the sweat pores." Therefore, cells related to skin such as umbilical vein endothelial cells and fibroblasts are closely connected with Defensive-Qi which is used to protect human from the pathogens outsides and similar to the definition of immune system. Defensive-Qi is also called Surface-Qi. The word "surface" is relative to internal viscera such as kidney and liver. As for cells such as lymphocytes that work for immune system, they are obviously related to Defensive-Qi.

**Discussion**

Since the reprogramming efficiency data of somatic cells are derived from different experiments, slight differences in experimental design may affect the accuracy of the results. In this review, all samples cited here are chosen as similar as possible in experimental design. Since the efficiency rates of each group of somatic cells differ by around one order of magnitude, consistent with previous research, these results may suggest the validity of the experiments cited in this paper. Whether the discovered pattern is still applicable to other cell sources need to be further identified with new experiments.

The regular pattern in reprogramming efficiency elucidated by TCM viscera theory may have predictive intention. Based on the findings in the YECM, cell types such as eye cells, saliva glands cells and periosteum cells belong in the kidney group. If their efficiencies match the pattern of those cells in their category, they probably have higher efficiency than that of cells in the group of liver and defense-Qi. As expected, later research shows that the periosteum has much higher reprogramming efficiency than that of fibroblast [48]. This finding is consistent with the regular pattern discovered. Therefore, this pattern might be another new angle for people to theoretically predict the reprogramming efficiency.

**Conclusion**

In sum, we have categorized somatic cell types by their reprogramming efficiency rates in this review, which displays an interesting regular pattern between the TCM theory and the reprogramming efficiency of different somatic cells in the retrovirus system with OSKM transcription factors. An alternative perspective on cell properties and the connection between Chinese Tradition Medicine with iPS cells field may appear with further illustration. Moreover, this finding may lead to the prediction of reprogramming efficiency of new somatic cell candidates. Probably, it may contribute to the database of current reprogramming efficiency as well.

**Vision**

Although the regular pattern appeared is summarized above, due to the time constraint, based on the current data, the strict proof for cause and result relation can't be deeper explored in this study.

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