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EDITORIAL

Perspectives of gene combinations in phenotype presentation

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Abstract

Cells exhibit a variety of phenotypes in different stages and diseases. Although several markers for cellular phenotypes have been identified, gene combinations denoting cellular phenotypes have not been completely elucidated. Recent advances in gene analysis have revealed that various gene expression patterns are observed in each cell species and status. In this review, the perspectives of gene combinations in cellular phenotype presentation are discussed. Gene expression profiles change during cellular processes, such as cell proliferation, cell differentiation, and cell death. In addition, epigenetic regulation increases the complexity of the gene expression profile. The role of gene combinations and panels of gene combinations in each cellular condition are also discussed.

Key words: Gene combination; Cellular phenotype; Stem cell; Cancer stem cell; Gene expression

Core tip: This article discusses the effects of gene combination on cellular phenotype, demonstrating different capacities for proliferation and differentiation. Recent advances in genome technology have revealed that each cell type exhibits different gene expression patterns, suggesting that the combination of genes might represent different cellular features or phenotypes. Because cellular diversity is derived from the regulation of gene expression, it is important to investigate the genes in various cells, including differences between cell types or the processes of cell development. Moreover, because stem cells are used as therapeutics, these gene combinations should be evaluated for the assembly of cells in medicinal use.

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INTRODUCTION

Recent advances in genome technology have revealed several perspectives associated with gene regulatory networks[1]. Databases, such as encyclopedia of DNA elements (ENCODE), use chromatin immunoprecipitation and high-throughput sequencing to describe human transcription factors associated with cell type differences[1]. Recently, stem cells, such as induced pluripotent stem (iPS) cells, have been developed for medical application. For safe application, researchers must address concerns about the ability of stem cells to switch the cellular phenotype to cancer or other cellular phenotypes different from those originally expected. There are also arguments concerning the immunogenicity of iPS cells[2-4]. Thus, the clarification of stem cell characteristics is required for the safe application of iPS cells. There is little evidence indicating that mutations do not frequently occur during reprogramming[5]. In this review, gene combinations associated with cellular phenotype, particularly in stem cells, are discussed to address the possibility of the detection of cell features using gene combinations to facilitate the safe application of stem cells without tumorigenesis.

From the viewpoint of the risk of tumorigenesis associated with stem cells and the origin of cancer, genes regulated in cancer stem cells are also discussed.

VARIETY OF GENE REGULATION

Genes involved in self-renewal and pluripotency

Cell self renewal is defined as cellular duplication, whereas pluripotency is defined as differentiation into any cell type, including their own, a process in which genes, such as *Pou5f1* (also known as *Oct4* or *Oct3/4*), *Sox2*, *Klf4*, or *Nanog*, play an important role. A previous study indicated that combination of Oct4, Sall4, and Nanog is involved in transport, metabolism, and development in mouse embryos[6]. *Pou5f1* (*Oct4*) is a key factor in the maintenance of embryonic stem cells. In addition to *Pou5f1* (*Oct4*), several gene combinations and coordinated network gene regulation are essential to stem cell development. DNA methyltransferase 3B (*Dnmt3b*) has also been implicated as a developmental stage determinant working in conjunction with other pluripotency factors[6].

Cell cycle control and long-term self-renewal have been linked, and the cyclin-dependent kinase inhibitor 1A (P21) (*Cdkn1a*) has been shown to regulate *Sox2* gene expression and adult neural stem cell expansion[7]. The combination of p21, Sox2, and p53 is important for the regulation of neural stem cells, during which p21 binds to Sox2 enhancers and negatively regulates the gene expression of *Sox2*, and increased expression of Sox2 in *p21*-null neural stem cells induces p53-dependent growth arrest[7]. Reprogramming clonally expanded antigen-specific $CD8^+$ T cells obtained from an human immunodeficiency virus (HIV)-1-infected patient to pluripotency using the gene combination of *POU5F1* (*OCT3/4*), *SOX2*, *KLF4*, and *c-MYC* resulted in the expression of the pluripotency markers SSEA-4, Tra-1-60, and Tra-1-81, and the re-differentiation of these cells into $CD8^+$ T cells resulted in T-cell functionality and antigen specificity, as observed in the original cytotoxic T lymphocyte clone[8]. A study demonstrated that lactic acid bacteria-incorporated human dermal fibroblast cell clusters were positive for the multipotency markers *NANOG*, *POU5F1* (*OCT3/4*), *SOX2*, and SSEA-4, and these cells were converted into multipotent cells that differentiate into multiple lineages[9]. Interestingly, not all pluripotent markers were expressed in the lactic acid bacteria-incorporated cells[9]. A minimum gene combination might be sufficient for the acquisition of multipotency. A recent report has revealed that protein kinase C, located downstream of fibroblast growth factor-2 (FGF-2), regulates the self-renewal of human pluripotent stem cells[10].

Genes involved in proliferation

The tumor suppressor p53 is associated with metabolism and senescence[11]. p53 suppresses the gene expression of the tricarboxylic acid cycle-associated malic enzymes, including $NADP^+$-dependent, cytosolic malic enzyme 1 (*ME1*) and *ME2*, leading to the regulation of cell metabolism and proliferation[11]. The combination of p53 and malic enzymes has been implicated in cellular senescence. Another finding demonstrated that the fifth component (CSN5) of the mammalian COP9 signalosome complex binds to CDK2 and controls senescence via cytoplasmic cyclin E[12].

Genes involved in differentiation and stem cell activity

The combination of force and the scleraxis (*SCX*) gene promotes the commitment of mesenchymal stem cells derived from embryonic stem cells to tenocytes[13].

The differentiation of T cells is regulated through various genes, including the regulation of Th17 cell differentiation through sphingosine 1-phosphate receptor 4 (*S1PR4*), an endothelial differentiation, G-protein-coupled (*EDG*) receptor[14]. Glycerophosphodiester phosphodiesterase 2 (GDE2) induces spinal motor neuron differentiation[15]. GDE2 also inactivates the Notch activator RECK (reversion-inducing cysteine-rich protein with kazal motifs) through a glycosylphosphatidylinositol anchor cleavage and inhibits the Notch pathway, which promotes neurogenesis[15]. The cyclin-dependent kinase inhibitor $p27^{Kip1}$ has been implicated in the regulation of cellular processes, such as T cell differentiation[16]. Moreover, the regulation of CD8 T cell memory through $p27^{Kip1}$ has also been proposed[16].

WNT signaling is one of the major signaling pathways involved in the regulation of bone homeostasis[17]. WNT-β-catenin signaling is essential for the commitment of bone marrow-derived cells to the osteoblast lineage, and this signal inhibits adipogenic and chondrogenic differentiation[17]. Molecules, such as low-density lipoprotein receptor-related protein 5 (LRP5), frizzled (FZD), adenomatous polyposis (APC), and GSK-3β, are involved in WNT signaling[17].

The endodermal differentiation of human embryonic stem cells is regulated through interactions between integrin receptors and extracellular matrix proteins[18]. In addition to growth factors, such as FGF, TGF-β, and WNT, extracellular matrix proteins, such as fibronectin (FN) and vitronectin (VTN), are involved in the differentiation of the embryonic stem cell population[18]. The combination of extracellular matrix proteins and integrins might affect the phenotype of stem cells during development.

It has recently been shown that fatty acid synthase (Fasn) is involved in the proliferative activities of adult neural stem and progenitor cells[19]. Fasn is a key enzyme involved in *de novo* lipogenesis, and the deletion of Fasn in mouse neural stem and progenitor cells impairs adult neurogenesis[19]. A recent study has revealed that Notch inhibition induces cochlear hair cell regeneration[20].

MOLECULES AND GENES INVOLVED IN STEM AND iPS CELL DEVELOPMENT

Molecules and genes involved in stem cell development

Various sources of autologous stem cells are used for the application of cell-based therapies. Mesenchymal stem cells (MSCs) differentiate into various cells, such as osteogenic cells, adipocytes, and chondrocytes. The markers

commonly expressed in human adult MSCs include alanyl (membrane) aminopeptidase (ANPEP, CD13), integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) (ITGB1, CD29), CD44 molecule (Indian blood group) (CD44), intercellular adhesion molecule 1 (ICAM1, CD54), 5'-nucleotidase, ecto (CD73) (NT5E), Thy-1 cell surface antigen (THY1, CD90), endoglin (ENG, CD105), activated leukocyte cell adhesion molecule (ALCAM, CD166), and HLA-DR[21]. Proteins, such as CD34 molecule or vascular cell adhesion molecule 1 (VCAM1, CD106), are either positive or negative markers in MSCs depending on the conditions[21,22]. The combination patterns of these molecules might be different in each MSC phenotype, reflecting the cell source or developmental stage. The gene expression profile and differentiation or proliferation capacity is altered at each stage of MSC culture[23]. The gene expression of EPH receptor A5 (*EPHA5*) and nephroblastoma overexpressed gene (*NOV*) is upregulated, whereas the expression of necdin homolog (mouse) (*NDN*) and runt-related transcription factor 2 (*RUNX2*) is downregulated during late-stage MSC culture compared with early-stage culture[23]. The MSCs in different environments might exhibit unique phenotype, as demonstrated through the capacity of these cells for differentiation or proliferation.

The leucine-rich repeat-containing G-protein-coupled receptor 5 (*Lgr5*) is a Wnt target gene and a stem cell marker for the small intestine, colon, stomach, and hair follicles[24]. The regeneration of $Lgr5^+$ liver stem cells is mediated through Wnt signaling[24]. *LGR5* might become a good marker for self-renewing stem cells in combination with Wnt signaling pathway genes.

High-throughput screening of small molecules that inhibit human pluripotent stem cells has identified 15 compounds that specifically inhibit stem cell development[25]. This approach contributes to the safe application of stem cells in cell therapy, as undifferentiated cells have a risk of forming tumors, and these molecules might play a role in the safety of cell therapy in regenerative medicine.

Genes involved in iPS cell development

Recent advances in technology have been developed to investigate iPS cells. A study showed that iPS cells successfully differentiate into the intermediate mesoderm[26], and pluripotent stem cells can be transformed into Odd-skipped related 1 (*OSR1*)-expressing intermediate mesoderm[26]. These OSR1-positive intermediate mesoderm cells form nephrogenic tissue cells[26]. Marker genes are typically used to detect stem cell properties for the characterization of pluripotent stem cells[27]. Alkaline phosphatase (AP), Pou5f1 (Oct4), Nanog, Sox2, Tra-1-60, Tra-1-81, SSEA3, and SSEA4 are markers for the undifferentiated state of human embryonic stem cells[27].

iPS cells are reprogrammed through four factors [Pou5f1 (Oct3/4), Sox2, Klf4, Myc (c-Myc)][28-30]. A recent study has shown that the absence of *Cdkn1b* (*p27*) enables cellular reprogramming through only two factors, *Oct4* and *Klf4*[31]. Considering that CDKN1B is a tumor suppressor, and that the absence of *Cdkn1b* increases *Sox2* expression, reprogramming might be associated with proliferation, and the presence of factors associated with cancer progression or the absence of factors associated with cancer repression are important for inducing the reprogramming of iPS cells. Recent studies have revealed that the AMP-activated protein kinase (AMPK) agonist metformin (Imidodicarbonimidic diamide, *N*,*N*-dimethyl-) regulates the expression of cancer stem cell-specific genes and inhibits the tumorigenicity of iPS cells[32]. This effect is mediated through the metformin-induced death of cancer stem cells[33,34].

An association between threonine and S-adenosylmethionine metabolism has been demonstrated in pluripotent stem cells, resulting in the regulation of histone H3 lysine 4 trimethylation[35]. Upon the epithelial differentiation of iPS cells from fibroblasts obtained from ectodermal dysplasia-related patients with mutations in the DNA-binding domain of the *p63* gene, APR-246 ($PRIMA\text{-}1^{MET}$), a small compound that restores the function of mutant P53 in human tumor cells, rescues the differentiation potential of these cells[36,37]. Recent studies using patient-specific iPS cells have shown that the induction of adult-like metabolic energetics from peroxisome proliferator-activated receptor gamma (PPAR-γ) activation is involved in the pathogenesis of arrhythmogenic right ventricular dysplasia/cardiomyopathy[38]. In this model, gene mutations in plakophilin-2 (*PKP2*) are important factors for the regulation of plakoglobin nuclear translocation and β-catenin activity[38]. A mathematical model for tumor evolution has revealed that somatic mutations in the cancers of self-renewing tissues exist before tumor initiation, which might provide an interesting interpretation of the relationship between stem cells and cancer initiation[39].

MicroRNA involvement in cell development

It has been recently shown that microRNAs play important roles in cell development. For example, the correlation between microRNA expression profiles and cancer survival has been investigated[40]. The involvement of the miR-200/ZEB2 pathway in Oct4/Sox2-induced mesenchymal-to-epithelial transition and iPS cell generation has also been shown[41].

MOLECULES AND GENES INVOLVED IN CANCER DEVELOPMENT

Genes involved in cancer development

MDM2 oncogene, E3 ubiquitin protein ligase (MDM2) and Mdm4 p53 binding protein homolog (mouse) (MDM4) (MDMX) proteins are deregulated in human cancers and induce cancer progression through inhibition of the tumor suppressor p53 (encoded by *TP53*)[42]. The $Mdm2^{-/-}$ or $Mdmx^{-/-}$ mice are embryonically lethal, whereas conditional knockout mice exhibit apoptosis in the central nervous system, intestine, or heart. MDM2 overexpression induces carcinoma; however, the expres-

sion of MDM2 does not accelerate the onset of cancer progression in transformation-related protein 53 (*Trp53*)-null mice[42]. The combination of MDM2, MDMX, and p53 signaling is one of the important pathways for cancer development. Wnt and β-catenin signaling is activated in cancer cells[43]. EMT and Hedgehog signaling is also important for cancer cell phenotypes, such as differentiation states or malignancies[44-46]. Notch signaling is also involved in cancer, and Notch signaling genes have been associated with cancer patient survival rates[47]. Cyclin plays important role in cell proliferation and cancer development[48-52], and cyclin D1 expression might also be involved in cell growth in gastric cancer[53]. The reduced expression of claudin in breast cancer mediates the development of cyclin $D1^{low}/ID1^{high}$ tumors[54]. Peroxisome proliferator-activated receptor (PPAR) also plays an important role in cancer[55]. Thus, the origin of cancer from stem cells suggests the importance of investigating molecules expressed in stem cells in cancer.

Genes involved in cancer stem cells

Several studies have been conducted to identify cancer-associated genes, and evaluations of sets of cancer-associated genes have revealed a variety of biological functions for these genes[56]. Cancer genes have been analyzed from several viewpoints, such as common Pfam domain analysis in Cancer Gene Census, which demonstrated that the protein kinase domain is the most common cancer gene-encoding Pfam domain (http://www.sanger.ac.uk/genetics/CGP/Census/analysis.shtml). This annotation scheme and the cancer gene assessment project showed that transcription factors and regulators are the largest functional classes[56]. Many genes involved in stem cell development are classified as transcription factors, and the genes activated in cancer stem cells might be included in these Cancer Gene Census analyses. Many studies have been conducted to reveal the origin of cancer cells[57]. It has been shown that SmoM2, KRas, or p53 is involved in epithelial skin cancer[57]. Intestinal stem cells expressing Bmi1 stabilize β-catenin expression and form intestinal adenomas, whereas *APC* deletion in transit-amplifying cells induces microadenomas[57]. Cancer stem cell markers include CD44, EpCAM, EZ112, Notch-1, Nanog, and Oct4[58]. Recent studies have shown that the dual expression of Lgr5 and Dclk1 is observed in cancer stem cells[59,60]. The Jun dimerization protein 2 (*Jdp2*) regulates the *Trp53* promoter and negatively regulates *Trp53* expression[61]. As previously demonstrated, the loss of *Trp53* promotes tumorigenesis, and common insertion sites were identified in $Trp53^{+/+}$, $Trp53^{+/-}$, and $Trp53^{-/-}$ mice[61]. Tumors retaining a wild-type copy of *Trp53* exhibit common insertion sites in several genes, including *Jdp2*[61]. Tumor growth is not always defined through the expression of a single common cancer stem cell marker, highlighting the importance of gene combinations in clarifying cancer stem cell phenotypes. These insights were derived from a study indicating that cells do not express previously identified CSC markers in each cancer (CD34 for acute myeloid leukemia, CD44 for breast cancer, CD133 for glioblastoma and colon cancer, CD271 for melanoma) and have the capacity for tumorigenesis[62].

The epithelial-mesenchymal transition (EMT) is one of the important factors in cancer metastasis[63]. Recent studies have shown a close association between the origin of cancer stem cells and EMT[64,65]. The induction of EMT through Twist, Snail, and TGF-β exhibits a similar phenotype as cancer stem cells, and EMT and cancer stem cell signatures share the same cell population in nonresponders[66]. Factors promoting EMT in complex with Smad, include Snail1, Zeb1, Zeb2, Lef1/TCF, β-catenin, AP-1, SP1, and HMGA2[66]. The homeobox factor *PRRX1* is an EMT inducer and a biomarker associated with patient survival and the lack of metastasis[67]. The upregulation of Prrx1 is required for the initiation of metastasis, whereas the downregulation of Prrx1 is important for secondary tumor colonization[67]. Another study indicated that the substitution of Twist1 is essential for tumor metastases[68]. Twist1 expression induces EMT, and the downregulation of Twist1 might be important for secondary tumor formation by circulating cells[68]. Twist signaling associates EMT with stemness[69-71]. Twist1 interacts with Bmi1, a member of polycomb-repressive complex 1 (PRC1), which maintains chromatin silencing[72,73]. *BMI1* is involved in the self-renewal of neuronal, hematopoietic, and intestinal cells through the repression of the *INK4A-ARF* locus[72,73]. During EMT, various networks are regulated, including the interaction of SNAI with the *CDH1* promoter (which encodes E-cadherin)[74]. SNAI2, ZEB1 and ZEB2, E47, KLF8, and Brachyury are involved in EMT, which represses E-cadherin and other junction proteins, such as claudins and desmosomes[74]. Moreover, TWIST1, forkhead box protein C2 (FOXC2), goosecoid, E2-2 (TCF4), homeobox protein SLX1, and paired mesoderm homeobox protein 1 (PRRX1) are also involved in EMT, characterized by reduced E-cadherin expression and increased vimentin expression[74].

CONCLUSION

In summary, gene combinations are useful for distinguishing cell populations or evaluating cellular characteristics. The cellular phenotype is regulated through genes and epigenetic modifications. A deep understanding of cellular features is important for the safe medicinal application of cells, including pluripotent stem cells. A panel of gene combinations to evaluate and predict precise cell phenotypes is essential for the future development of the cell field. Thus, systems or databases to provide the context of the panel of gene combinations might be of great value for the appropriate assessment of these cells.

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