Differentiated and undifferentiated states

Characteristic differences between differentiated and undifferentiated states

Control of many processes related to the cell fate is provided by some extrinsic and intrinsic factors that posses different appearances in different states of the cell; whether differentiated or undifferentiated. Besides, it seems that there are some close correlations between these factors which are discussed in the following. I n Fig. 8. a schematic of some differences between two states, differentiated and undifferentiated, has been shown.

Transcriptional factors

Transcription factors such as Oct4, Nanog, c-Myc, and Sox2, as intrinsic factors are able to influence SC fate, and express the differences between differentiated and undifferentiated cells. Expression of a complex network of these factors has been appeared to maintain self-renewing undifferentiated state in SCs [1-6] . Developmental controlling in the mammalian embryo [3, 7-8] can be possible by these regulatory genes, some of which such as Oct4 that regulates the transcription of other genes [9] . Oct4 is a member of POU proteins consisting of six classes that are divided based on the DNA binding domain homology [10-12] . Participation of Oct4 in the cellular processes such as metabolic [11, 13-14] and develomental regulation [14-17] can be occurred through target genes which encode transcription factors. It should be noted that Oct4 can activate or repress these genes; maintenance of these genes in an inactive state is correlated with Oct4 [18] .

Pluripotency, self-renewality, and differentiation of SCs [2-3, 5, 19-22] are crucially connected to the expression of Oct4. It has been reported that

pluripotency is remained in ESCs with a normal level of Oct4 [20, 23] , and low levels of it can cause ESCs to become trophectodermal cells [9, 24] . Deficiency of Oct4 in mouse embryos has caused differentiation into trophectoderm and failure to form the inner cell mass (ICM) [5] . While extra embryonic mesoderm or endoderm lineages can be created from ESCs with high levels of Oct4 expression [9, 24] . In fact, this factor is essential for the maintenance of self-renewal state in theICMof the blastocyst [5, 8] ; and during embryonic development, its expression has been seen in all blastomeres [7, 9] . In addition to Oct4, Nanog has also been shown to play an effective role in pluripotency of ICM cells [6] . Surprisingly, these factors are able to reprogram somatic cells to induced pluripotent stem cells (iPSCs) [12, 18, 25-27] , which are the SCs that are generated in the laboratory under reprogramming condition by which mature adult cells can be reprogrammed to an ESC-like state [28-29] . These cells are capable of differentiating into all three germ layers (endoderm, ectoderm, mesoderm), like an ESC [12] .

x [30-31]

x [32]

There are many studies indicating Oct4 as a common requirement in the reprogramming [33-36] . Moreover, it has been shown that Oct4 is one of the three proteins which are sufficient to reprogram differentiated adult cells to the ESC lineage, in mouse and human [11, 31, 33-34] . It should be implied that Oct4 acts as a marker not only in normal SCs, but also incancer SCs [21-22] .

Signaling pathways

Cell fate is closely correlated with the signaling pathways like Notch, Wnt and Shh, as a part of cellular microenvironment [37-38] . Inhibition of differentiation and increase in the cell numbers by induction of proliferation, which are two critical elements protecting the concept of self-renewal, can be altered through these pathways. In the zebrafish embryos, increased levels of Notch signaling has caused a reduction in the number of endothelial cells, while the number of cells increased when the levels of Notch signaling decreased [39] . In HSCs, it has been revealed that this signaling is down-regulated with differentiation and acts as a critical parameter in the maintenance of undifferentiated states of these cells [40-42] . It has been shown that inhibition of Notch signaling can lead to the accelerated differentiation of HSCsin vitro [41] . According to Duncan et al. and Calvi et al., not only osteoblasts but also Jagged/Notch signaling activated by these cells, might be important ways of regulating self-renewality in HSCs. In other words, maintenance of homeostasis in adults, has been reported via participation of Notch signaling system [38, 43] . Moreover, it has been demonstrated that forced activation of this pathway not only increases self-renewality of HSCs, but also immortalizes primitive hematopoietic progenitor cells [38, 40] . Moreover, not only in HSCs, but also in the other cells like pancreatic progenitors, implication of this pathway in cell maintenance has been shown [44] .

Another signaling pathway mentioned above is Wnt. Wnts, a family of protein ligands, can be expressed in a wide range of tissue types and influence

many processes such as tissue homeostasis, embryogenesis, and generation of cell polarity [40, 45-46] . It has been seen that activity of this pathway is required to control spindle orientation, for example in C. elegans [47] . Additionally, this pathway is involved in oxidative phosphorylation (OXPHOS) [48-49] .

Besides, this pathway can act as a niche factor; it plays a regulatory role in the maintenance of self-renewing state, for example in HSCs [38, 40, 45-46, 50-51] . Some findings suggest that Notch signaling with Wnt signaling companionship may be able of influencing self-renewality of HSCs [38, 41] . Based on the studies, over-expression of activated forms of β-catenin [46] , which is a mediator in the Wnt signaling pathway, can expand immature cells like HSCs, and promote self-renewal capacity of them, while over-expression of Wnt signaling inhibitors, such as axin enhances degradation of β-catenin, which causes HSCs to lose their ability to repopulate [40, 45, 51] . Additionally in skin, Wnt signaling pathway has shown a significant role in promoting SC activation and expansion [37] .

β-catenin also plays a crucial role in cell-cell adhesion. An interaction between β-catenin and E-cadherin has been reported. It has been proposed that induction of β-catenin release from the membrane into the cytoplasm can be occurred by repression of cadherin [45, 52] . It should be implied that Wnts have a relation with the required genes during self-renewal; Wnt has shown a repressing role on Nanog, besides overexpression of Oct4 has caused an increase in β-catenin transcriptional activity [46] .

Wnts can be also related to thereceptortyrosine kinases (RTKs) which are the high-affinitycell surface receptorsfor some niche components such asgrowth factors, cytokines, andhormones, and can send signal through these reseptors. During the growth of tissues, Wnt signals play a controlling role on the expression of these receptors and their ligands in order to provide the balance between proliferation and differentiation [46] . Meanwhile change in this pathway has been shown to relates to on/off switching in the expression of telomerase reverse transcriptase (TERT) [53] , which is a catalytic subunit of theenzymetelomerase, as a pluripotency marker.

Surprisingly, Wnt signaling pathway can paly role in cancer [45, 50, 54] . Indeed, activation of Wnt signaling is involved in the maintenance and growth of not only SC, but also cancer cells. It means that there is a dual nature in the self-renewal signals, as deregulation of Wnt signaling is reported to be linked to the tumorigenesis [54] .

Another interaction between self-renewal factors refers to the Shh signaling pathway. It can induce the expression of Bmi1, Sox2, and N-Myc by which an increase in the proliferation of neural precursors has been reported. Bmi1 can act as a required factor for self-renewal of SCs [27, 55] . Trans-differentiation of mouse fibroblasts into NSC-like cells has been reported to be in association with Bmi1. Moreover, reprogramming of fibroblasts can be associated with this factor in combination with Oct4 [27] .

Energy metabolism and mitochondrial factors (metabolic cues)

Existence of the energy depending processes in the living systems is an important property of them. In fact, all cells need energy to function, and this energy is provided through metabolic pathways consisting of two main categories; anabolic and catabolic pathway. Energy is produced in the catabolic pathways, while biosynthetic pathways, anabolics, are energy consumer. Respiration is one of the important catabolic pathways, and occurs in the cells with aerobic conditions. The first step in the aerobic respiration, glycolysis, is performed in the cytoplasm. The second, which is tricarboxylic acid cycle (TCA cycle), occurs in the mitochondrial matrix, and the third is OXPHOS that generates adenosine tri-phosphate (ATP), the energy carrier, and carries out in the mitochondrial inner-membrane [56] .

Mitochondria is the main source of energy metabolism and ATP production [57-60] . These organelles produce ATP through an active process, as mentioned OXPHOS. During mitochondrial OXPHOS, an electron transportation with proton (H^+ ions) translocation across the mitochondrial inner-membrane take place [60] . This process which results in oxygen consumption, H_2O making, and ATP production is performed by a series ofprotein complexesto create a mitochondrial membrane potential (Δάμ m) via pumping protons out of the mitochondrial matrix into the inner-membrane space [61] . Indeed, electrons are carried fromelectron donors(such asNADH) toelectron acceptors(such asO_2) in theredox reactionsbyelectron transport chains(ETC), containing three energy-transducing enzymes; NADH dehydrogenase (Complex I), Coenzyme Q – cytochrome c reductase(Complex III), andcytochrome c oxidase(COX or Complex IV) (Fig. 9). In fact, these

reactions release energy to transport protons across the mitochondrial inner-membrane [60] .

Movement of protons produce anelectrochemical gradientacross the membrane, the proton-motive force (pmf). The pmf expressed in units of electrical potential, consists of âˆ†áµ m and âˆ†pH which is the pH gradient, the concentration difference of protons across the mitochondrial inner-membrane [60] . It should be implied that, under most conditions âˆ†áµ m dominates pmf. This potential which influences almost all activities in the mitochondria, is accounted to be 150–180 mV [60] . Mitochondria also involves in many cell processes like differentiation, apoptosis and metabolism of key cellular intermediates, and also plays a key role in controlling Ca^{++} concentration [1, 57, 62-64] . It is known thatendoplasmic reticulum(ER) has a critical role in Ca^{++} signaling and in fact is a significant storage site for calcium [65] . The physical association between mitochondria and ER not only indicates Ca^{++} signaling role of the mitochondria, but also corroborates that mitochondria can play a temporary role in Ca^{++} storage [65] .

It has been suggested that function and integrity of mitochondria may influence the viability, proliferation and differentiation potentials of SCs [1, 62, 66] . Based on the studies about mitochondrial features in differentiated and undifferentiated states, the relationship between mitochondrial localization/morphology and stemness maintenance seems reasonable [67-69] . As in low passage cell cultures of primate adult stromal cells, which are a form of mesenchymal stem cells (MSCs) derived from the adipose tissue,

perinuclear clustering of mitochondria has been reported [61] . These perinuclear arrangements can also act as cues in cancer cell populations. Based on the evidence , the perinuclear arrangement of mitochondria reported as a similarity between SCs and cancerous states, highlights mitochondrial functions in common features between these two groups of cells [1, 69-70] .

Like perinuclear arrangement, reduced mitochondrial number has been observed in undifferentiated SCs. There are many studies which corroborate this result [48, 61-62, 67-72] . Moreover, low mitochondrial DNA content might act as an indicator of stemness. Up-regulation of ESCs' capacity to transcribe mitochondrial DNA and increased mitochondrial DNA copies per cell have been reported in committed ESCs with lost pluripotency. In fact, there is a close correlation between SC pluripotency and mitochondrial DNA [69-70, 73] . Besides, low levels of ATP have been reported in undifferentiated mouse and human ESCs [48, 72] . In ESCs undergoing differentiation, mitochondrial activity and also mitochondrial DNA replication increases, which represent that mitochondrial DNA encodes ETC components. Thus an increased ATP content occurred with increased mitochondrial activity might act as a signal to initiate differentiation and causes the stemness to decrease [57, 68-70] .

In cancer cells, ATP generation through OXPHOS shifts to ATP generation through glycolysis. Indeed, a reprogramming in metabolic pathways of these cells occurs by which glycolytic signal pathways are activated. This shift is known as the Warburg effect that happens even under normal oxygen concentrations [57, 59] . Glycolysis is an oxygen-independent process to

produce energy by metabolism of glucose and other sugars into pyruvate. Whereas, OXPHOS is an oxygen-dependent metabolism [57, 59] . In the presence of oxygen, an aerobic respiration will take place and the pyruvate is produced. While in the absence of oxygen, that is an anaerobic condition, fermentation of the pyruvate will occur.

Importantly, metabolic characterization shows that SCs prefer anaerobic metabolism [57, 61] . The studies on the mouse epiblast SCs, has shown that respiration activity of mitochondria is lower in these cells, in contrast to the earlier stage mouse SCs [74-75] . This metabolic transition is correlated with the activity of certain genes controlling mitochondria. As during the transition from ICM to epiblast cells, these gene are turned down [61] . Based on the studies, undifferentiated cells are more glycolytic than differentiated ones. During differentiation of ESCs, it has been reported that production of energy is switched to OXPHOS [48] ; OXPHOS plays an essential role in differentiation of ESCs into cardiomyocytes and creation of sarcomeres [61, 76] . Moreover, more glycolytic nature of undifferentiated cells has also been seen in adult SC, like HSCs [77] and MSCs [61, 78] which demonstrates high rate of glycolysis and high levels of lactate production , in contrast to differentiated ones. It is known that OXPHOS is related to the mitochondrial DNA transcription, as mitochondrial mass and oxygen consumption increase with differentiation in order to keep OXPHOS, while limited transcription has been reported in stemness situation [1, 57, 67-69, 73] .

During the differentiation of ESCs, the physiological changes in mitochondria like changes in the mitochondrial DNA copy number, ATP content, and

oxygen consumption take place associated with the down-regulation of Oct4, Sox2 and Nanog, while in undifferentiated state of SC, mitochondrial activity is suppressed [1, 73] . In fact, the decision between oxidative and anaerobic glycolytic metabolism can be mediated by these genes and their products [13, 18] . A connection between several common Oct (Oct4, Oct1) targets and metabolism has been reported [18, 79] ; as low mitochondrial function and high rates of glycolysis have been shown as characterizations of the metabolic pattern in which Oct1 association has been seen [18] .

In addition to the mentioned mitochondrial factors, âˆ†áµ m, a reflection of mitochondrial functional status, seems to be able to associate with the differentiation status in cells, tumorigenicity and malignancy [1] . In mitochondria, control of the bioenergetic parameters such as the redox state of pyridine nucleotides are linked to âˆ†áµ m or pmf. The pyridine nucleotides, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), participate in energy transduction and OXPHOS as electron carriers. They arepresent in two forms; oxidized (NAD^+, $NADP^+$) and reduced (NADH, NADPH) forms. It has been revealed that pyridine nucleotides as well as the balance between their two forms play essential role in some functions such as maintenance of redox status, regulation of ion channel, and cell signaling under not only normal condition but also pathological condition [80] .

âˆ†áµ m in differentiated cell like fibroblasts can be maintained through ETC and form proton gradient within this chain. Whereas in human pluripotent

SCs this maintenance has been reported through glycolysis. It should be noted that âˆ†áµ m is maintained by ATP hydrolysis when ETC in differentiated cells is impaired [81] . Moreover, in leukemia cells cultured on the bone marrow–derived mesenchymal stromal cells, an increase in aerobic glycolysis and reduction in âˆ†áµ m have been reported. It has also been suggested that the Warburg effect in these cells is promoted through mitochondrial uncoupling [82] . Mitochondrial uncoupling occurring under physiologic conditions, is defined as the abrogation of ATP synthesis in response to âˆ†áµ m, and is mediated by uncoupling proteins (UCPs) which are mitochondrial inner-membrane proteins regulating cell metabolism. In cancer cells, their increased dependency on glycolysis has been suggested to be possibly related to the inability of these cells to synthesize ATP in response to âˆ†áµ m, and not the fact that these cells are unable to reduce oxygen [82] .