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Mini Review

Investigating the role of Sirtuins in cell reprogramming

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Abstract

Cell reprogramming has been considered a powerful technique in the regenerative medicine field. In addition to diverse its strengths, cell reprogramming technology also has several drawbacks generated during the process of reprogramming. Telomere shortening caused by the cell reprogramming process impedes the efficiency of cell reprogramming. Transcription factors used for reprogramming alter genomic contents and result in genetic mutations. Additionally, defective mitochondria functioning such as excessive mitochondrial fission leads to the limitation of pluripotency and ultimately reduces the efficiency of reprogramming. These problems including genomic instability and impaired mitochondrial dynamics should be resolved to apply cell reprograming in clinical research and to address efficiency and safety concerns. Sirtuin (NAD+-dependent histone deacetylase) has been known to control the chromatin state of the telomere and influence mitochondria function in cells. Recently, several studies reported that Sirtuins could control for genomic instability in cell reprogramming. Here, we review recent findings regarding the role of Sirtuins in cell reprogramming. And we propose that the manipulation of Sirtuins may improve defects that result from the steps of cell reprogramming.

Introduction

Cell reprogramming techniques have emerged with novel techniques to treat a variety of human diseases in the regenerative medicine field (1). In the reprogramming process, 'immortality' is regarded as a key to develop rejuvenation strategies (2). Takahashi et al. stated that cell reprogramming using four transcription factors such as Oct4, Sox2, Klf4, and c-Myc could convert terminally differentiated cells into induced pluripotent stem cells (iPSCs) (1). The pluripotency of iPSCs has opened up numerous possibilities for regenerative medicine to treat many diseases (3). Despite the powerful ability of iPSCs to treat numerous diseases, major concerns in recent iPSCs research include enhancing reprogramming efficiency and genomic stability. Genomic instability in iPSCs is generated in several steps of the cell reprogramming process (4). Cellular reprogramming goes through an intricate process that is similar to biological pathways of tumorigenesis (5). The essential factors for cell reprogramming are associated with tumorigenesis. For example, c-Myc and Klf4 play central roles in tumorigenesis, and Oct4 acts as an important initiator for germ cell tumors (5). In addition, to inducing changes in the original cell identity, cell reprogramming needs reactivation of the telomerase to continue to survive (6). Maintenance of telomere as an enzyme for telomere elongation is important for genomic stability during reprogramming (7). Telomerase is reactivated during reprogramming and the length and epigenetic state of the telomere contributes to rejuvenation in iPSCs. Shortening of the telomeres influences the reprogramming efficiency and the quality of the iPSCs (8). The strategy to solve the genome instability in cell reprogramming research for application in disease modeling and clinical cell therapy (9). During cell reprogramming, cells experience a metabolic shift into the glycolytic state (10). Oxidative stress and DNA damage from the cell reprogramming process results in a metabolic imbalance (11). Because of these metabolic shifts, mitochondrial activity is hampered and cannot react when energy is demanded due to cellular respiration. The reduction of mitochondrial activity during cell reprogramming is a matter that should be resolved for increasing iPSCs efficiency. Sirtuins known as histone deacetylases are relevant to the control of longevity, energy metabolism, and cell development in mammals (12). It was reported that sirtuins can affect the fate of stem cells through deacetylation of histone and non-histone proteins involved in gene expression (13). Recent studies demonstrated that the deficiency of Sirtuins influences reprogramming efficiency (14) and contributes to genomic instability, which as we noted, is an important issue in the cell reprogramming process (15).

Here, we review evidence on the significant role of Sirtuins in the cell reprogramming process.

Genomic instability in cell reprogramming

Genomic instability occurs during the cell reprogramming process (16). A number of studies report that after reprogramming iPSCs exhibit the genomic abnormalities such as chromosomal aberrations (17). Because of the transcription factors used in cell reprogramming cells have an increased risk of both tumor formation and genetic mutation (18). Telomerase is significantly upregulated during cell programming (8). Pluripotent cells show high activity of telomerase responsible for synthesizing telomeres in the reprogramming process (19). The iPSCs generation process showed that telomerase reverse transcriptase was upregulated in cells during cellular reprogramming (1). Telomerase activity and telomere length affect the state of pluripotency (20). In cell reprogramming, reactivation of telomerase has been shown to promote efficiency of iPSC reprogramming by maintaining telomere length and self-renewal potential for a relatively long time (21). Upon reprogramming, telomere lengthening is affected by a decrease of DNA methylation (22) and a reduction of methylation in histone H3 at lysine 9 (H3K9) m3 and histone H4 at lysine 20 (H4K20) m3 (8). Some studies investigated the differences in the telomere dynamics during reprogramming (21). Telomere shortening is a crucial issue in reprogramming process in that it hampers sufficient iPSCs generation. During the cell reprogramming process the proliferation rate increases causing replication stress and genomic structural variation (23). Additionally, recent studies show that pluripotent stem cells have an abnormal cell-cycle regulation such as a shorter G1 phase. The ataxia telangiectasia mutated Rad3 (ATR)-mediated checkpoint pathway is an essential replication stress response that generates genomic instability during reprogramming (24). Other studies report that Checkpoint kinase 1 (CHK1) overexpression could enhance both the reprogramming efficiency and the iPSCs quality (25). Abnormal cell cycle regulation is a distinct feature and the control over it is considered important to current reprogramming research. Accordingly, to realize the application of iPSCs in clinical research, we need a comprehensive understanding of genetic instability and should find an appropriate solution for it in cell reprogramming.

Mitochondrial dynamics in cell reprogramming

Mitochondria is a multifunctional organelle and plays a crucial role in many cellular

mechanisms such as energy production, apoptosis, reactive oxygen species (ROS) production, senescence, and metabolism (26). Mitochondrial homeostasis has been shown to be essential for maintenance of a pluripotent state. Ji et al. report that a decrease of ROS production in the mitochondria could improve iPSCs quality (27). Also excessive mitochondrial fission and knockdown of the mitochondrial DNA polymerase could trigger a lack of pluripotency (28). Tricyclic antidepressant (TCA)-derived cytosolic acetyl-CoA is essential for maintaining histone acetylation and an open chromatin state during cell reprogramming (29). Reprogramming somatic cells into iPSCs triggers impairment of the mitochondrial network during the reprograming process (30). Besides, during cell reprogramming, cells show particular characteristics including immature and globular mitochondria (31), and poorly developed cristae (32). Reduced expression 1 (REX1) known as a pluripotency marker regulates cell fate through its effect on mitochondrial dynamics (33). The knockdown of Dynamin-related GTPases-1 (DRP1) triggers the elongation of the mitochondrial network (34) and regulates membrane dynamics in a variety of cellular mechanisms and in mitochondrial fusion (35). One study demonstrated that the DRP1-GTPase inhibitor impedes cell reprogramming of human fibroblasts to iPSCs. The mechanistic target of rapamycin (mTOR) promotes cellular homeostasis and multiple signaling events that affect reprogramming (1). Inhibition of mTOR leads to an immediate decrease in mitochondrial respiration (36) and subsequently influences the generation of iPSCs (37). Taken together, cell reprogramming influences abnormal mitochondria function and homeostasis, and mitochondrial dynamics should be a focus for future cell reprogramming research.

Sirtuins in genomic instability derived from cell reprogramming

Sirtuins as an NAD+-dependent histone deacetylase have been involved in the improvement of longevity and metabolism in mammals (38). Given that histone acetylation is associated with gene activation (39), Sirtuins act as an epigenetic regulator of gene expression by histone deacetylation (40). Sirtuins have been shown to be essential for the silent chromatin state of the ribosomal RNA genes and telomeres. In mammals, Sirt6 has been reported to maintain telomeric chromatin and to enhance replicative capacity (41). According to cell reprogramming research the activation of Sirtuins considerably enhances the efficiency of cell reprogramming (42). Several studies demonstrate that the inhibition of histone deacetylases leads to increases of histone acetylation levels, chromatin opening, and ultimately could enhance efficiency of cell reprogramming (43). Sirtuins could possibly

control the chromatin state by modulating the activation of enzymes such as H4K16Ac (44) and H3K4me3 that can upregulate cell reprogramming. Sirt1 is intimately linked to the maintenance of human embryonic stem cells pluripotency by inactivating p53 (45). Besides stem cells derived from Sirt6, knockout mice cells exhibit expression of Oct4, Sox2 and Nanog and present Sirt6's function in balance between pluripotency and differentiation (46). Sirt1 could lead to the deacetylatization of Sox2 (14) and Sirt1's overexpression induces the demethylation of the Oct4 promoter (47) and also affects reprogramming efficiency. Myc stability, important in cell reprogramming, could also be regulated by Sirt2 (48). Sirt1 deacetylates c-Myc by interacting physically with the C-terminus of c-Myc (49). Sirt1 induces p53 translocation into the mitochondria (50) and modulates Nanog expression (51) and is an important reprogramming factor. Judging by the metabolic state of the cell, Sirt1 can affect the epigenome change and the activity of chromatin-modifying enzymes (52). Sirt1 histone deacetylase regulates the epigenetical change and gene expressions in cells by translating a metabolic shift in the reprogramming process (53). A recent study showed that Sirt6 inhibits the transcription of Hypoxia-inducible factors (HIF1)-alpha and Myc (54). Sirt6 is essential for the maintenance of the telomere position in cells (55) and the deficiency of it leads to DNA damage and genomic instability (15). In addition, Sirt6 protects cells against stress by repairing DNA damage and preserving telomere integrity and controlling metabolic homeostasis (56). Sirt6 can deacetylate lysine 9 on histone H3 (H3K9Ac) (41) and lysine 56 on histone H3 (H3K56Ac) (57). And Sirt6 can recruit the chromatin remodeler Sucrose Nonfermenting Protein 2 Homolog (SNF2H) (58). As we have seen, Sirtuins influence cell reprogramming efficiency by regulating the activities of histone deacetylases, by controlling the chromatin state of telomere, and by being involved in metabolic shifts during cell reprogramming (Figure 1).

Activators and inhibitors of Sirtuins.

Several compounds are known to be activators of Sirtuin. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), SRT1720, Oxazolo [4,5-b] pyridines derivative, imidazole [1,2-b] thiazole derivative, and 1,4-dihydropyridine (DHP) derivatives are typical compounds that are known activators of Sirtuins (59). The exact mechanisms of Sirt1 activation by these activators is still unclear, but many of them seem to activate Sirt1 through allosteric activation, particularly, the resveratrol mediate activation of Protein Kinase AMP-Activated Catalytic Subunit Alpha 2 (AMPK), which is an initial sensor that increases NAD+ levels

leading to activation of Sirt1 (60).

The metabolic effects of Resveratrol, the most common Sirtuin activator, relate to the cAMP level elevation in muscles (61). Also the general health in mice fed with a high caloric diet improved and they showed a marked reduction in sings of aging (62). These results open the possibility of clinical use of commercial micronized Respiratory Syncytial Virus (RSV) formulation, SRT501, for lowering blood glucose and improving insulin sensitivity in patients with type 2 diabetes (63). Moreover, SRT1720 has been shown to induce cell death in multiple myeloma cells (64) and significantly decrease tumor growth in a preclinical evaluation for cancer treatment (65). Also, as a new activators of Sirt1 unrelated to Resveratrol, a series of oxazolo pyridines was identified for potential therapeutic targets to treat different diseases (66). For example, compound 29 showed antidiabetic activity in types 2 diabetes (67) and SRT2104 was tested in a clinical trial of patients with metabolic inflammatory (68) and cardiovascular diseases (69).

In contrast, Splitomicin, HR73, Sirtinol, AGK2, Cambinol, Salermide, Tenovin, and Suramin are inhibitors of Sirtuin (70). The reaction mechanism of Sirtuins is the cleavage of nicotinamide (NIC) from NAD+ whereas ADP-ribose binds its acetyl-peptide with the formation of an o-alkylamidate intermediate. Sirtuin inhibitors hamper cleavage of NIC from NAD+. Suramin is an especially potent inhibitor of Sirt1, Sirt2 and Sirt5 (71). It inhibits NAD+-dependent deacetylase activity with an IC50 value of 22uM leading to mitochondrial dynamics disruptions (72). Several studies revealed that pharmacological inhibition of Sirtuin1 by Sirtinol inhibits prostate cancer cell proliferation in which Sirtuin1 is highly enriched (73). Moreover, Salermide, a sirtinol derivative, induces cell death via inhibiting MAP kinases erk1/2, p38 and JNK paring Sirtuin1 and Sirtuin2 in various human cancer cell lines derived from leukemia, lymphoma, colon, and breast primary malignancies (74). 6-Chloro-2,3,4,9-tetrahydro-1 H-Carbazole-1-carboxamide (EX527) is also known as a Sirtuin1 inhibitor and EX-527/SEN0014196 reduced neuronal death caused by mutant Huntington proteins in cell-based assays in preclinical studies of Huntington's disease (75). More importantly, activation and inhibition of Sirtuin by small molecules is a complicated process and the effects of activation and inhibition of Sirtuin occasionally depend on the physiological state of the specific cells for its activity. For instance, increased activity of SIRT1 after treatment with resveratrol in the immediate immune response reduced the NFkB activation in the NFkB-dependent inflammatory genes in microglia and neuronal loss (76). This suggests that Sirt1 is working as anti-inflammatory mediator, whereas decreasing Sirt1

activity by sirtinol potentiates inflammatory responses, presumably occur via Sirt1-mediated deacetylation of p65 (77). Moreover, unlike Sirt1 effects on the inflammation, Sirt6 activity is positive for a given pro-inflammatory gene expression upregulating Tumor necrosis factor alpha (TNFa) and Interferon Production Regulator (IFNr) synthesis on both innate and adaptive immune cells (78). Such complexity of sirtuin activity in the various physiological states of cells lead to the difficulties of Sirtuin activators or inhibitors in determining the desired outcome of cell reprogramming efforts.

Sirtuins in mitochondria dynamics during cell reprogramming

Mitochondrial dynamics are controlled by many cellular proteins such as fission proteins DRP1 (79), fusion proteins Mitofusins 1 and 2 (Mfn1/2) (80), and optic atrophy 1 (OPA1) proteins (81). The mitochondrial network was reported to have changed during the cell-cycle progression and mitosis processes (82). Mitochondrial distribution during mitosis acts in a critical role during asymmetric cell division in stem cells. Mitochondrial fusion, fission, and biogenesis are linked with mitochondrial dynamics as well (83). One study showed that mitochondrial fission and fusion contributes to the maintenance of pluripotency (84). Loss of mitochondrial fusion proteins such as Mfn1/2 (85) leads to a metabolic transition by activating HIF-1alpha signaling in iPSC reprogramming (86). Sirt1 can exert nuclear localization signals and nuclear export signals and can come and go between the cytoplasm and the nucleus (87). Sirt3 regulates the activity of both mitochondrial enzymes (88) and mitochondrial biogenesis through activation of the Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-alpha) (89). OPA1 is a GTPase anchored to the mitochondria's inner membrane and is linked to the maintenance of mitochondria crista structure and protection of cells against stimuli (90). Sirt3 has been known to bind directly to OPA1 and subsequently modulates mitochondrial dynamics (91). Sirt1 can enhance mitochondrial function by involving PI3K/Beclin 1 and mTOR signaling (92). Additionally, Sirt1 can destruct damaged mitochondria through a mitophagy process (93). Mitophagy is dependent on the activities of specific factors such as PTEN-induced putative kinase 1 (PINK1) and E3 ubiquitin ligase Parkin (94). According to genetic research, Sirtuins affect mitophagy by inhibiting mitochondrial defects in PINK1-null mutants (95). In addition, Sirt1 suppresses the activity of the HIF1-alpha (96) that inhibits mitochondrial function. Sirt3 is known as a powerful regulator of the ROS detoxification via deacetylation of Mangano-Superoxide Dismutase (MnSOD) in mitochondria (97). Sirt3 eliminates excessive ROS

production through activation of a Forkhead box O3 FOXO3-alpha (98) and then regulates mitochondrial dynamics (99). Proceeding from what has been said above, Sirtuins may affect cell reprogramming efficiency through the regulation of mitochondrial dynamics including the regulation of fission proteins, the regulation of mitophagy, the modulation of mTOR signaling, and the control of ROS production (Figure 2).

Perspectives and conclusions

In conclusion, cell reprogramming has limitations including genomic instability and impaired mitochondrial dynamics. Until now, the appropriate solution to overcome these limitations was not fully investigated. Sirtuins contribute to genomic stability and mitochondrial dynamics through several signaling reactions and the activation of enzymes. After examining the roles of Sirtuins, we propose further research should look at the multiple other functions of Sirtuins in cell reprogramming. We suggest investigating more advanced manipulation of Sirtuins in cell reprogramming and ultimately expect to promote more efficient and safe cell reprogramming processes and technology.

Abbreviation list

Sirt: Sirtuin

iPSCs: induced pluripotent stem cells

ATR: Ataxia telangiectasia mutated Rad3

CHK1: Checkpoint kinase 1

ROS: reactive oxygen species

TCA: Tricyclic antidepressants

REX1: Reduced expression 1

DRP1: Dynamin-related GTPases-1

mTOR: The mechanistic target of rapamycin

HIF1: Hypoxia-inducible factors

SNF2H: Sucrose Nonfermenting Protein 2 Homolog

DHP: 1,4-dihydropyridine

AMPK: Protein Kinase AMP-Activated Catalytic Subunit Alpha 2

RSV: Respiratory Syncytial Virus

EX527: H-Carbazole-1-carboxamide

TNFa: Tumor necrosis factor alpha

IFNr: Interferon Production Regulator

Mfn1/2: Mitofusins 1 and 2

OPA1: Optic atrophy 1

PGC1-alpha: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PINK: PTEN-induced putative kinase 1

MnSOD: Mangano-Superoxide Dismutase

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Conflicts of Interest

The author declares no conflicts of interest.

Figure legends

Figure 1. The function of sirtuins on genome stability

Sirt 1, 2, and 6 control the chromatin state by regulating the activation of enzymes during chromatic remodeling. Sirt1 removes acetylates in Sox2 and Myc removes methylates in Oct4. Also, Sirt2 modulates the stability of the Myc protein. SIRT6 can deacetylate H3K9Ac and H3K56Ac and is involved in the transcription of c- Myc. AC: Acetylation, ME: Methylation, SIRT: Surtuin.

Figure 2. The relationship between sirtuins and mitochondrial dynamics caused by cell reprogramming

Cell reprogramming leads to mitochondrial dynamics such as changes in fission and fusion. The mitochondrial dynamics are linked with the maintenance of the pluripotent state. Sirtuins regulates mitochondria fission by binding with fission proteins such as OPA1 proteins.

Also, sirtuins promote mTOR signaling, the activity of PGC1-alpha, and ultimately eliminate ROS production during cell reprogramming. mTOR: The mechanistic target of rapamycin, OPA1: Optic atrophy 1, PGC1-alpha: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS: reactive oxygen species.

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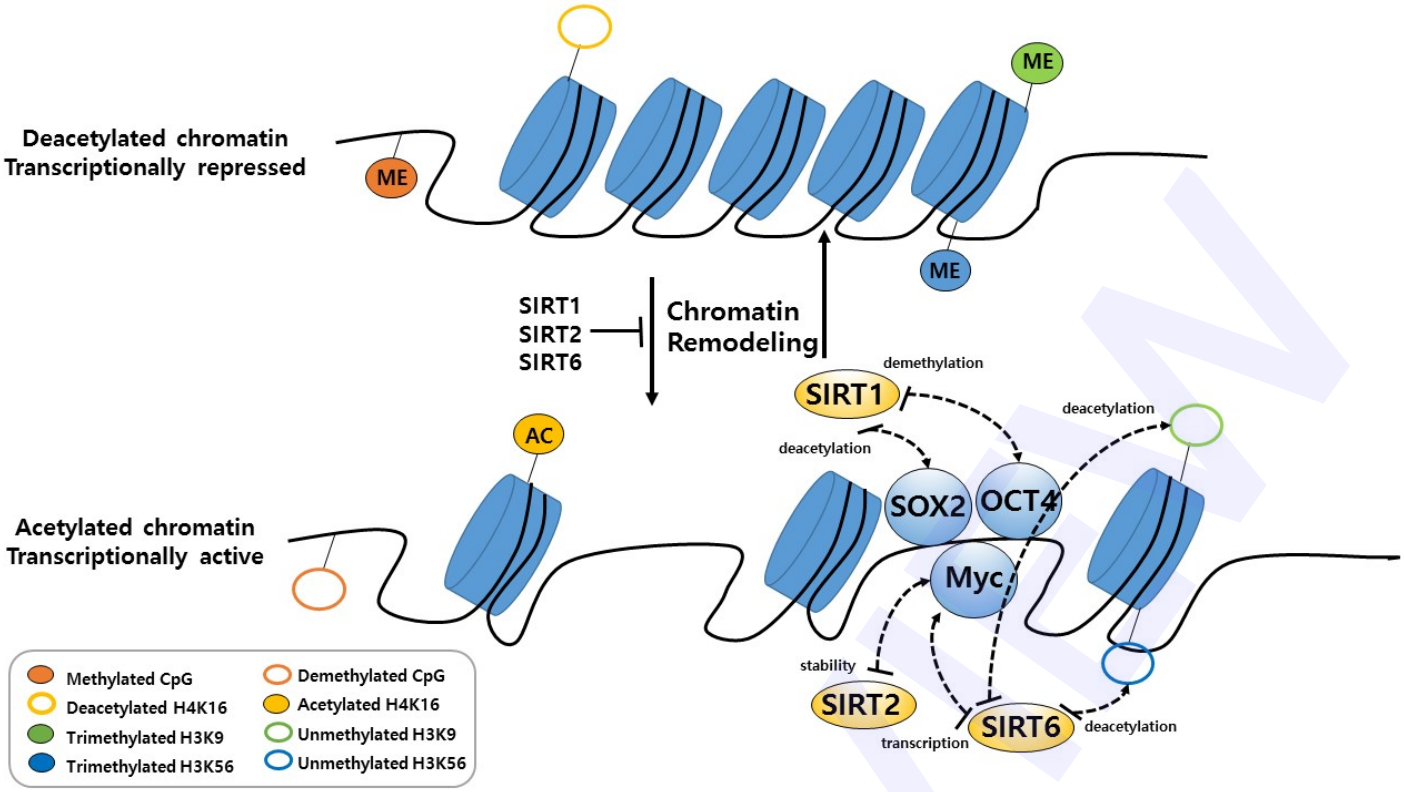

Fig. 1.

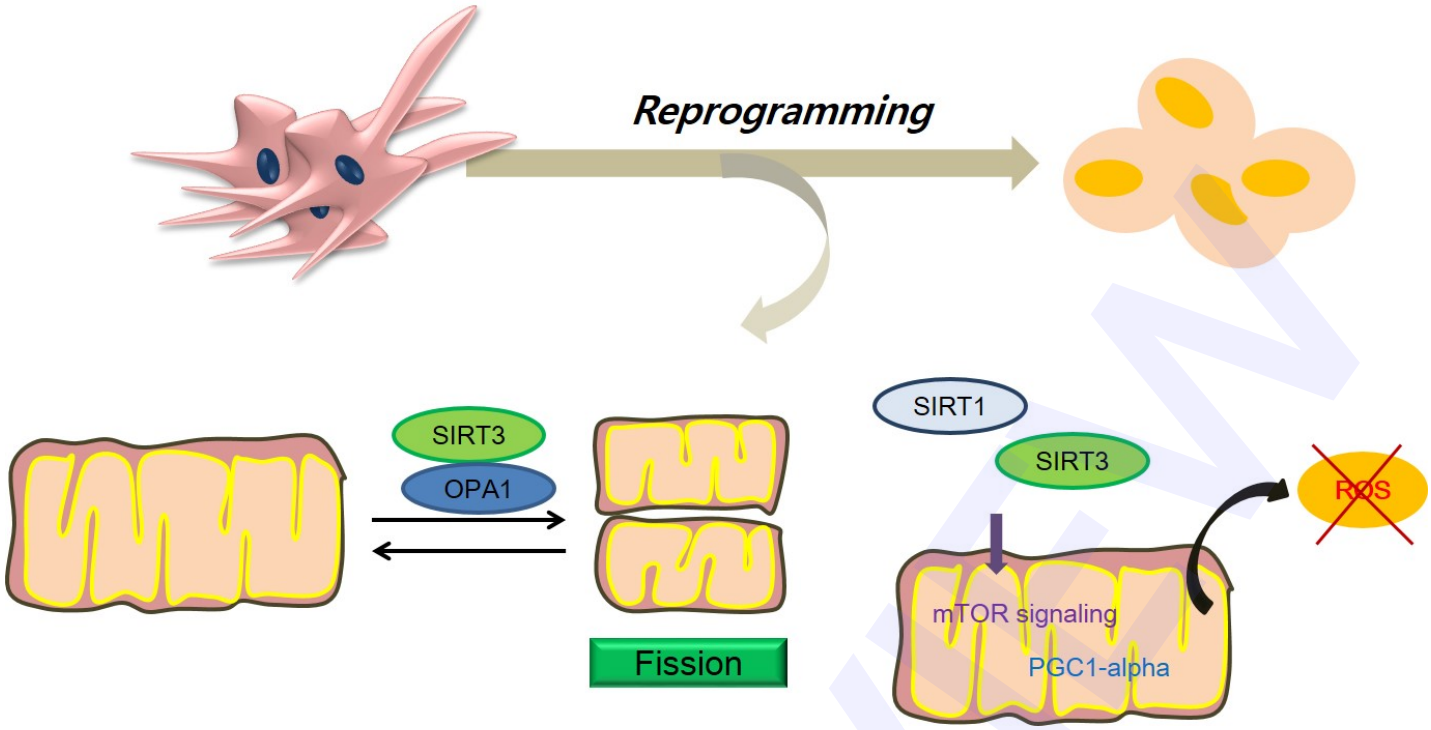

Fig. 2.