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Review

Nuclear receptor 3B (NR3B): Bridging mitochondrial reprogramming and pluripotency through crosstalk with Nanog

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ABSTRACT

Tumor-initiating cells (TICs) have been identified as cells that account for tumor heterogeneity. Recent studies demonstrated that genes controlling stem cell biology play key roles in maintaining TICs and promote their development into cancer. Here, we review the function of nuclear receptor 3B (NR3B), a transcriptional factor that orchestrates mitochondrial function and its recently discovered roles in pluripotency. In particular, we summarize the crosstalk between NR3B and Nanog, the "stemness gene" as well as how NR3B and NR3B-regulated mitochondrial functions may command the function of Nanog as a pluripotency factor.

KEYWORDS: NR3B, Nanog, tumor-initiating cells (TICs), mitochondrial reprogramming

INTRODUCTION

Mitochondria play key roles in the alteration of cellular respiration, lipid and glucose metabolism, energy status, oxidative stress, and apoptotic stimuli [1]. Up until very recently, relatively little was understood of the differences and importance of mitochondrial function in progenitor cells vs. their differentiated progenies. It was reported that adult primate stromal cells at early passages have a higher oxygen consumption rate (OCR) and a lower ATP/mitochondrial DNA content when compared with long-term cultured cells [2], suggesting that mitochondrial metabolic activity is related to cell differentiation. Recent studies primarily using embryonic stem cells (ES) or induced pluripotent stem cells (iPSCs) have revealed a role of mitochondrial function in cell fate determinations [3]. Pluripotent stem cells largely display immature mitochondrial structures and respiration function with low mitochondrial DNA copy numbers. However, reprogramming iPSCs requires an initial induction of mitochondrial respiration to ensure successful reprogramming.

In tissue progenitors, low mitochondrial DNA (mtDNA) contents, reactive oxygen species (ROS) level and reduced oxidative phosphorylation (OXPHOS) have been reported [4, 5]. Inhibition of mitochondrial functions leads to expansion of hematopoietic stem cell (HSC) pools whereas inducing mitochondrial function through activation of the molecule mammalian target of rapamycin (mTOR) impairs their maintenance [1, 2]. However, high membrane potential typically associated with enhanced mitochondrial respiration and function is also associated with stemness [6, 7]. In addition, asymmetric distribution of mitochondria is observed during division of stem cells. It is found that the daughter cell where stemness needs to be maintained

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inherits more new mitochondria with enhanced function [8]. Furthermore, expression of genes related to glucose uptake, oxidative phosphorylation, and fatty acid β-oxidation is higher in ovarian tumor-initiating cells (TICs) compared to differentiated ovarian cells, indicating a higher ability to direct pyruvate towards the tricarboxylic acid (TCA) cycle [9]. Glioma TICs have a higher mitochondrial reserve capacity compared to the differentiated cells [10] whereas breast TICs exhibit higher ATP content compared to its differentiated progeny [11]. Glioblastoma TICs also depend on OXPHOS for their energy production and survival [12]. Thus, the existing studies indicate an inconsistent role of mitochondria in stemness and differentiation, suggesting that its role in the maintenance of progenitor cells or TICs maybe cell type and context-dependent.

In the liver, abnormalities of mitochondrial function are common occurrences with liver diseases including cancer [13-15]. These defects include altered expression of genes involved in mitochondrial respiratory complexes, mtDNA mutations, abnormal production of ROS, as well as increased or decreased mitochondrial numbers. Human liver cancer displays progenitor cell signatures, indicative of the presence of TIC populations [16, 17]. The presence of TICs accounts for the high cellular heterogeneity observed in hepatocellular carcinoma (HCC), challenging therapeutic targeting [18]. Using CD133+ TICs isolated from the Huh7 human liver cancer cell line, it was recently discovered that OXPHOS was negatively regulated by Nanog in TICs, which depended on fatty acid oxidation (FAO) for energy [19]. Using HCC cells isolated from a liver cancer model where deletion of tumor suppressor *Pten* (Phosphatase and Tensin homologue deleted on chromosome 10) leads to cancer following fatty liver development [20-23], we have shown that the increased OCR and FAO are correlated with the transformation potential of the tumor cells [24]. Decreasing OCR by inhibiting the expression of NR3B1 (nuclear receptor 3B1), a nuclear transcription factor controlling global mitochondrial transcription, results in reduced transformation potential.

In this review, we will focus on new, timely findings for the mitochondrial function in progenitor cell regulation with a focus on pluripotency and liver TICs (Box 1). We will discuss how NR3B family members, which are master regulators of mitochondrial functions, contribute to the maintenance of stemness. Particularly, we will discuss how NR3B interacts with Nanog and the implications of such an interaction on the role mitochondria may play in stemness regulation of TICs. We will also discuss our recent discoveries on a potential role of Nanog, a "stemness factor" in the regulation of mitochondrial functions.

1. NR3B, a family of transcriptional factors that orchestrate mitochondrial function

The limited encoding capacity of mitochondrial DNA makes it necessary for the nuclear origin of regulatory factors governing mitochondrial gene expressions. Two families of nuclear transcriptional factors, nuclear respiration factors (NRF1 and NRF2) and NR3B (NR3B1, NR3B2 and NR3B3), are characterized for their roles in the regulation of the global transcription of genes involved in mitochondrial function [25, 26]. Mitochondrial gene transcription involves the binding of mitochondrial RNA polymerase on the promoters of mitochondrial genes in association with Tfam, which unwinds mitochondrial DNA, and TFB1M or TFB2M, which are mitochondrial transcriptional factors. All four proteins (polymerase, Tfam, TFB1M and TFB2M) are encoded by nuclear DNA and move to the mitochondria to regulate mitochondrial transcription. In the nucleus, NR3B and NRFs are the two major transcriptional factors that regulate the expression of these four and other mitochondrial genes encoded by nuclear DNA. While NRF1 and 2 directly bind to the promoters of Tfam and TFB2M to control biogenesis, the target genes of NR3B span both biogenesis and OXPHOS. NR3B also directly regulates the expression of NRF1 and NRF2. NR3B1 binds to genes encoding nearly all enzymes involved in glycolytic and pyruvate metabolism as well as the TCA cycle.

1.1. NR3Bs as regulators of mitochondrial respiration

The NR3B family of transcriptional factors are orphan nuclear receptors that are abundantly expressed in highly oxidative organs and have been recognized as the key regulators of adaptive energy metabolism [26]. NR3B1 was the first

- Recent studies have highlighted a role of mitochondrial function in cell fate determinations. Pluripotent stem cells largely display immature mitochondrial structures and respiration function with low mitochondrial DNA copy numbers. However, reprogramming iPSCs require an initial induction of mitochondrial respiration to ensure successful reprogramming.
- Existing studies indicate an inconsistent role of mitochondria in stemness and differentiation of adult progenitor cells. Its role in the maintenance of tumor-initiating cells (TICs) and tumorigenesis maybe cell type and context dependent.
- Expression of Nanog, a "stemness factor" is correlated with mitochondrial function fluctuations. A role for Nanog in mitochondrial transcription was recently reported and this function of Nanog was found to play a role in maintaining TICs in the liver.
- NR3B is a transcriptional factor that orchestrates mitochondrial response. NR3B is highly expressed in tumors and found to transform tumor cells and NR3B expression supports tumor transformation.
- NR3B2 was identified to be a better prediction marker for successful reprogramming of somatic cells to iPSCs. Expression of NR3B isoforms can replace Nanog to reprogram somatic cells to iPSCs.
- NR3B2 was identified as a Nanog regulated gene and it binds to Nanog and Oct4 to regulate gene expression.
- A NR3B-SOX2 motif has been identified for pluripotency maintenance, similar to the OCT-SOX motif.

Box 1. Highlights of novel key points.

NR3B family member identified using the DNA-binding domain of estrogen receptors as a screening probe [27]. For this reason, NR3B1 is also termed estrogen receptor-related receptor (ERR) but it lacks the ligand-binding domain of the estrogen receptor. Two other NR3B isoforms, NR3B2 and NR3B3, have been identified [27]. These factors bind to the consensus DNA sequence 5'-TCAAGGTCA-3', termed ERR response element (ERRE) and control a variety of metabolic processes [28]. NR3Bs are weak transcriptional factors by themselves. Both the activity and the expression of NR3Bs are significantly induced when they are bound by co-activators such as PGC-1 (peroxisomal proliferation activating factor γ coactivator-1) [29, 30].

NR3Bs play a predominant role in orchestrating mitochondrial transcription by either directly activating mitochondrial structural genes or indirectly activating major transcription factors governing mitochondrial biogenesis such as Tfam, TFB1M and TFB2M. Gene chip analysis shows that the gene network regulated by NR3B1 and NR3B3 is involved in all metabolic processes, particularly OXPHOS and lipid metabolism [31, 32]. *In vivo* analysis shows that each NR3B family member by themselves are dispensable during embryogenesis even though loss of NR3B2 is associated with embryonic lethality due to placenta insufficiency [32-34]. Mice lacking either NR3B1 or NR3B3 display deficiencies in adaptive mitochondrial functions. NR3B1 is particularly necessary for generating energy required in response to physiological and pathological stresses in multiple tissues. Mice lacking NR3B1 are lean and resistant to high-fat diet-induced obesity and also defective in adaptive thermogenesis [34]. NR3B3 is predominantly expressed in the cardiac tissues and required for the cardiac muscle to switch to oxidative metabolism after birth [32]. The *in vivo* function of NR3B2 in metabolism has not been extensively studied due to placenta deficiency [33]. Rescue experiments with tetraploid injection that allow the placenta to be developed from NR3B2 positive cells demonstrated that NR3B2 null embryos could develop normally. The effects of combined loss of all three or combination of two of the three NR3Bs have not been investigated.

1.2. NR3B's role in stem cells, cancer and tumor-initiating cells

Discovered as a potential isoform with homology to the estrogen receptor (ER) DNA-binding domain, studies on NR3Bs in early years had focused on their function in breast cancer development. NR3Bs prefer a single consensus half-site

5’-TnAAGGTCA-3’ instead of the tandem ER response element (ERE), 5′-GGTCAnnnTGACC-3′ that is required for the ER to bind to [28]. Evidence of NR3Bs binding to the endogenous ERE fragment is still lacking, even though NR3Bs can induce the activity of ERE-luciferase reporter construct [35]. A recent study using a promoter containing both an ERE and an ERRE showed that the ablation of ERRE but not ERE led to the loss of transcriptional regulation by NR3B [36], suggesting that there are specificities in the NR3B regulation of gene transcription. Gene array analysis using Affymetrix HG133A GeneChips on MCF-7 breast cancer cells only detected 15 out of 14,500 genes that are commonly regulated by NR3B1 and ER [37]. These studies together suggest that the NR3B target genes are not related to those regulated by ER regardless of the shared DNA-binding sequence. Nonetheless, these earlier studies established NR3B as a potential marker for cancers including breast, ovarian, cervical and prostate cancers [38-40]. In the liver, we have reported that the expression of NR3B is negatively correlated with that of PTEN, a tumor suppressor in human HCC specimens [41]. Analysis of the Oncomine database confirmed this analysis, showing that NR3B expression is higher in tumor than in non-tumor samples in 5 out of 6 HCC databases (data not shown).

Inspired by the early observation that cancer cells, unlike normal cells, display altered metabolic profiles, recent studies have highlighted the importance of metabolic regulation in tumor development [42]. These metabolic changes include alterations in glycolysis, OXPHOS, FAO, lipid biosynthesis and others. As the transcriptional factor orchestrating the mitochondrial metabolic response, NR3B has become an important factor at the crossroad of cancer and metabolism. It is recognized that tumor cells uniquely regulate glycolysis and OXPHOS to fulfill the need for bioenergetics, and NR3B isoforms contribute to these changes [43]. In breast cancer cells, NR3B was found to bind to the promoters of metabolic genes linked to cancer progression including lactate and isocitrate dehydrogenase genes. Blocking the transcriptional activity of NR3B1 altered the expression of these genes in MCF-7 cells. In MDA-MB-231 breast cancer cell line, introduction of small interfering RNA to downregulate NR3B1 also resulted in reduced growth rate of xenografted tumors [37]. Using a chemical inhibitor for NR3B, it was demonstrated that mitochondrial biogenesis is required for anchorage-independent survival and propagation of stem-like cancer cells [44]. In *Pten* null HCC cells, we showed previously that NR3B1 supports the enhanced OXPHOS that is concurrent with accelerated glycolysis [41]. Inhibiting the enhanced OXPHOS by targeting NR3B1 attenuated the ability of the cells to establish colonies, suggesting that the enhanced OXPHOS or NR3B1 is needed to support cell transformation.

Cancer cells are found to display characteristics similar to cells of embryonic origin that harbor high growth rate while changing cell fate. During development, drastic demand is placed on metabolism to accommodate the need for such rapid growth and differentiation into multicellular organisms. In the preimplantation embryo, totipotent stem cells depend on pyruvate metabolism and OXPHOS to sustain cell growth, and glycolysis is inhibited due to the relatively low hexokinase activity [1, 45]. After implantation, the embryo dramatically upregulates glycolysis and glucose uptake while undergoing the first round of differentiation [46, 47]. Consistently, embryonic stem (ES) cells established at this stage of development display high glycolysis rate. In stem cells, such a metabolic profile may allow energy production at a faster rate without the significant generation of ROS, which occurs with OXPHOS. However, maintaining proper mitochondrial function and integrity is also critical for pluripotency despite their dependency on glycolysis. High mitochondrial membrane potential is necessary for the ability of ES cells to form teratomas and maintain proper developmental potential [48-50]. Recent reports suggest that the induction of OXPHOS is needed to reprogram cell fate. It is reported that OXPHOS genes such as Tfam and NRF1 are induced during reprogramming of iPSCs [51]. Using single cell expression analysis, four transcriptional factors (NR3B2, Utf1, Lin28 and Dppa2) were found to be better predictors for successful reprogramming of iPSCs than those defined previously [52]. Further analysis shows that these four factors can efficiently reprogram

iPSCs, and the resulting iPSCs are able to contribute to the germline. NR3B2 was also found to be a direct transcriptional target of Nanog [53] and can replace c-Myc and Krüppel-like factor 4 (Klf4) to reprogram iPSCs with Oct4 and Sox2 [54]. This observation provoked the question of whether metabolic programming is necessary for reprogramming to be successful. Using NR3B1, NR3B3 and their cofactors to induce OXPHOS, it was demonstrated that at least an initial burst of the OXPHOS activity is necessary for the reprogramming process [55].

A common observation is that total ATP production is reduced in all progeny cells compared with hES (human embryonic stem) cells. While a switch to OXPHOS dependency for ATP production is often observed when stem/progenitor cells differentiate to more mature cell types [56], detailed analysis reveals more complicated energy metabolism that might be dynamically regulated. For example, hES cells generate 77% of their ATP through OXPHOS [57]. In neuronal stem cells differentiated from these hES cells, only 55% of total ATP production was generated by OXPHOS. The ratio for OXPHOS-derived ATP, however, was restored when the differentiation process was allowed for a prolonged period of time. Human fibroblasts (BJ Fibroblast) derived from normal foreskin also displayed lower OXPHOS rate (59%) than the hES cells. Thus, stem cells and cancer cells likely manipulate OXPHOS and glycolysis to balance their unique needs for energy production and building block availability. In cancer cells, such regulation may lead to high ROS production during rapid growth, leading to transformation. This can occur when glycolysis and OXPHOS are uncoupled, leading to both high glycolysis rate and high OXPHOS, like what we observed in the *Pten* null HCCs [41]. Similar uncoupling is also observed elsewhere [58].

2. Interaction of NR3B with Nanog, a pluripotency factor

Regardless of the different profiles of NR3B and ER-regulated target genes, earlier studies did demonstrate that NR3B1 and NR3B2 can both bind to EREs linked to the luciferase reporter and induce their expression [35]. Earlier transcriptional studies also demonstrated a role of NR3Bs in several genes that are induced by estrogen treatment [36], notably osteopontin, a gene involved in bone morphogenesis and recently demonstrated to be a potential marker for progenitor cells. In the liver, osteopontin-positive cells labeled with yellow fluorescence protein was found to participate in repopulation of injured liver induced by carbon tetrachloride and feeding of choline-deficient diet [59]. These studies indicated that NR3B might indeed be involved in maintaining the growth and identity of progenitor cells and particularly in the establishment of TICs. This view is further supported by recently reported roles of NR3Bs in iPSC reprogramming. However, literatures on how NR3B interacts with stemness are limited, though a role of metabolic programming in pluripotency has been recognized [3, 51, 56].

2.1. Nanog and mitochondrial function in stem cells

During culturing of mouse ES cells, leukemia inhibitory factor (LIF) is used to maintain pluripotency [60]. LIF acts through PI3K/AKT and JAK/AKT signals that both regulate Nanog expression. In addition, the TGFβ/BMP and Activin/Nodal signals have also been shown to be necessary for pluripotency in human ES cells [60]. Nanog is regulated by upstream signals including both PI3K/AKT and JAK/STAT3 in mouse ES cells and Activin/Nodal in human ES cells [61-63]. Forced expression of Nanog, bypassing LIF/Stat3 and BMP/Smad/Id pathways, is sufficient to maintain constitutive self-renewal in mouse ES cells [64]. It was found that the treatment using antimycin A, a chemical commonly used to inhibit complex III of the mitochondrial respiratory chain, can elevate the expression of Nanog [65]. This upregulation may result from a mitochondrial superoxide signaling which induces the expression of Nanog through the methylation of Nanog promoter [66]. Recently, our study showed that Nanog reduced mitochondrial OXPHOS and ROS production needed to maintain the self-renewal ability of TICs [19]. ChIP-seq analysis using the anti-Nanog antibody revealed the enrichment of promoter-proximal Nanog on genes associated with OXPHOS (*i.e.*, *Cox6a2* and *Cox15*) and fatty acid oxidation (FAO) (*i.e.*, *Acadvl*) [67]. The functionality of Nanog on these genes was supported by the enhanced production of mitochondrial ROS *via* Nanog silencing. In mice expressing the hepatitis C virus (HCV) NS5A gene [68-71], we showed

that the activation of Toll-like receptor 4 (TLR4) induced the expression of Nanog [41]. The HCV-TLR4-Nanog axis enhanced the genesis of TICs, hepatocarcinogenesis and chemo-resistance [41]. This result is further supported by the observation that Klf4 governs the transcriptional programming of mitochondrial biogenesis during cardiac development at the early postnatal period [72]. Klf4 is one of the two genes recognized as the prominent targets of Nanog [53] and plays key roles in pluripotency and reprogramming of iPSCs [61, 67]. These studies suggest that mitochondrial reprogramming may be important in cell fate determination, implying a role of NR3B in this process.

Nanog is a homeodomain-containing protein that was discovered during a screen for genes that could sustain self-renewal of ES cells without leukemia inhibitory factor (LIF) signal [64]. It forms homodimers through a tryptophan-rich (WR) domain and this dimer formation is necessary for its function as a pluripotency factor [73, 74]. Nanog, like Oct4 and Sox2, acts as a key regulator of pluripotency in both mouse and human ES cells [75-79]. Nanog, Oct4, and Sox2 constitute the core pluripotency circuitry and function together to regulate a significant proportion of their target genes in ES cells [80]. The vast majority of promoter regions bound by both Oct4 and Sox2 are also occupied by Nanog. Downregulation of Nanog *via* siRNA (small inhibitory RNA) in ES cells leads to a significant downregulation of Oct4 and loss of ES cell-surface antigens, and differentiation toward extra-embryonic endodermal lineages [64, 81], suggesting that Nanog sits at the top of the hierarchy for pluripotency. Nanog mRNA (messenger RNA) is present in pluripotent ES cells, and absent from differentiated cells [64]. In embryo, Nanog expression is first detected in morula, and it increases in the early blastocyst, and declines prior to implantation [81]. Nanog is also expressed in developing germ cells [82]. Nanog alone can induce differentiation of primed pluripotent epiblast stem cells towards the germ cell lineage [83]. Deletion of Nanog prevents acquisition of pluripotency in the inner cell mass of pre-implantation mouse blastocysts and renders mouse ES cells prone to differentiation [81]. The inner cell mass lacking Nanog cannot develop epiblasts [81]. Under defined cultured conditions, the constitutive expression of elevated levels of Nanog was found to sustain self-renewal of embryonic stem cells without LIF [84]. Forced expression of Nanog in cultured ES cells sustains pluripotency [64, 85-87]. Nanog also plays a crucial role in reprogramming. Nanog was one of the four transcription factors initially used to generate iPSCs from human somatic cells [88]. In the absence of Nanog, mouse somatic cells transfected with reprogramming factors failed to activate the full repertoire of pluripotency genes [89] and hence cannot be reprogrammed to iPSCs.

2.2. Crosstalk of NR3B with Nanog

Genome-wide mapping of *in vivo* protein-DNA interactions has identified many pluripotency genes bound by Nanog, including *Nr3b2*, *Foxd3*, *Rif1*, and *REST* [76]. Among these, Nr3b2 acts as a direct downstream target of Nanog that, when overexpressed, can replace the function of Nanog in the maintenance of pluripotency [53]. Nanog also physically interacts with multiple proteins that are involved in the maintenance of pluripotency, including Oct4, Sox2, NR3B2, NR3B1, and Sall4 [77, 90]. A key question in the understanding of pluripotency and cell fate determination is how these factors maintain pluripotency.

NR3B2 as a “stemness gene” is evidenced by its expression pattern in germ cells [26], as well as placenta defect and trophoblast proliferation associated with the null mice [33]. The role of NR3B2 as primary pluripotency transcription factors is further supported by its interlaced relationships with OCT4, SOX2 and Nanog, the three core pluripotency factors [76, 91]. NR3B2 was recently identified as a positively regulated target gene of Nanog using a genome-wide transcriptional array analysis [53]. As stated, expression of NR3B1, NR3B3 and their cofactors induces OXPHOS and this initial burst of the OXPHOS activity is necessary for the reprogramming process [55].

In Nanog expression-stimulated ES cells, NR3B2 transcription is stimulated due to enhanced binding of RNA PolII to *Esrrb*, the gene encoding NR3B2 [53]. This regulation is accredited to the mechanism by which Nanog regulates pluripotency as NR3B2 can replace Nanog to maintain pluripotency and

knockdown of NR3B2 results in spontaneous differentiation of the ES clones [76, 91]. Interestingly, NR3B2 is also found to co-immunoprecipitate with Nanog in ES cells [77, 90]. However, rather than synergize with Nanog, NR3B2 appears to antagonize gene transcription induced by Nanog. OCT4 is a characterized target of Nanog and a "stemness factor". Like Nanog, NR3B2 binds to the promoter of OCT4 and induces its expression. Overexpression of both Nanog and NR3B2 led to antagonistic effects on the Oct4 promoter rather than synergistic effects. The binding site for NR3B2 is near a putative binding site for Nanog. In addition, NR3B2 also directly interacts with OCT4 [92]. This interaction recruits NR3B to the close proximity of the OCT-SOX element on the Nanog promoter and positively regulates Nanog expression. In addition to the OCT-SOX motif, a NR3B-SOX2 motif with a gap of 2-8 basepairs has also been identified using ChIP-seq data [93].

Like NR3B, studies recently showed that Nanog is expressed in different types of human neoplasms, including germ cell tumors [94-97], breast carcinomas [97], osteosarcoma [98], pancreatic carcinomas [99], prostate cancer [100] as well as HCC [50]. Ectopic expression of Nanog induces an oncogenic potential in NIH3T3 [101]. However, Nanog alone is not sufficient to drive tumorigenesis. In an inducible model where Nanog is overexpressed in the mammary gland, it was shown that co-expression of Wnt-1 is necessary to promote tumorigenesis and metastasis [102]. In lung cancer cells, co-expression of Oct4 and Nanog increased the percentage of cells that are positive for CD133, an indicator for TICs [103]. In the liver, Nanog-positive TICs are induced by virus infection, alcohol and high fat consumption. In these TICs, Toll-like receptor 4 (TLR4) induces the expression of tumor-driver genes that collaborate with Nanog to induce tumorigenesis [104]. Whether NR3B can interact with Nanog to promote tumorigenesis is not known.

3. Perspective

In this review, we summarized recent findings on the potential function of mitochondrial reprogramming in the regulation of pluripotency and the maintenance of TICs with a focus on NR3B, a transcription factor characterized for its function in regulating mitochondrial biogenesis, OXPHOS, glycolysis and lipid metabolic processes. NR3B isoforms are found to play a role in pluripotency regulation and interact with Nanog both transcriptionally and through direct interaction. These recent findings

- Does mitochondrial reprogramming play a role in adult progenitor cell maintenance? During reprogramming of somatic cells, mitochondrial function has been shown to be important for cell fate determination. It is not clear if mitochondrial changes in adult tissues can indeed influence the differentiation and maintenance of tissue progenitors including tumor-initiating cells (TICs).
- Is the role of Nanog in liver TICs and cancer development dependent on mitochondrial functional changes? If so, which specific process influenced by Nanog plays a role?
- NR3B is a transcriptional factor that orchestrates mitochondrial response. NR3B is highly expressed in tumors and found to transform tumor cells. NR3B2 was identified as a Nanog regulated gene. Does NR3B expression mediate the function of Nanog in the maintenance of adult tissue progenitors and TICs? If so, which specific process influenced by Nanog plays a role?
- NR3B2 was identified as a core transcriptional factor for pluripotency. What about the other NR3B isoforms? What is the role of mitochondrial function in NR3B2-regulated pluripotency and maintenance of progenitor cells?
- How does the interaction between NR3B isoforms and Nanog differ among ES cells, iPSCs, tissue progenitors and TICs? How does the dynamic change of such interactions contribute to pathogenesis, i.e. tumorigenesis?
- How is the interaction between NR3B and Nanog regulated?

Box 2. Outstanding questions.

highlighted the importance of metabolism in cell fate determination. Particularly, metabolic reprogramming has long been recognized as an important feature of cancer cells. These recent findings reinforce this view and suggest that NR3B, through its interaction with Nanog, may play a key role in this regulation and in pathogenesis when these processes are deregulated. These discoveries also bring up new questions (Box 2) as to how this interaction is controlled and how such a regulation contributes to tumorigenesis. Furthermore, while the NR3B interaction with Nanog changes cell fate, which of the processes regulated by this interaction may contribute to cell fate determination is a crucial question that remains to be answered. New discoveries on how NR3B interacts with Nanog and other pluripotency factors will likely lead to a novel understanding of the biological and pathological processes that depend on cell fate determination.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest for any of the authors.

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