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Review Article

Transdifferentiation between bone and fat on bone metabolism

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Abstract: The transdifferentiation of bone and fat is a new insight in studying the increasingly bone marrow fat in the process of osteoporosis of elderly or menopause crowd which is increasing in prevalence. The loss of bone mass in osteoporosis is multifactorially determined and includes genetic, hormonal and environmental determinants. Although it has long been considered whether the transdifferentiation process does exist *in vivo* and whether it could be find in the same individual, interaction between skeleton and adipose tissue has been proved pre-clinically and clinically by increasing evidence. Here we focus on the current understanding of the transdifferentiation between bone and fat, the molecular interactions and future clinical implications of recent studies linking the transdifferentiation to bone metabolism diseases. Furthermore, a set of recommendations of bone and fat transdifferentiation on bone metabolism are also presented to facilitate evaluation of this magic process.

Keywords: Transdifferentiation, bone, fat, osteoporosis

Introduction

The concept transdifferentiation initially described the multipotency of adult somatic cells (ASCs) to differentiate into cell lineages different from the cell where the somatic stem cell exists, and even into cells which are originated from other germ layers [1]. Today developmental biologists use "transdifferentiation" to explain the ability of apparently fully differentiated cells derived from a certain tissue to change into cells that show characteristics of a different tissue in response to either cell culture or surgical removal of adjacent tissue [2].

Over the years, the common belief was that the tissue-residing ASCs were developmentally restricted to the specified lineages where they resided. The only dissention in mammals was pathological differentiations into tumor or cancer cells. However, the last few years have witnessed that some ASCs might transdifferentiate into totally different cell or lineage. In terms of bone marrow cells transplantation, lots of researches have showed the large expression of donor-derived reporter genes such as lacZ and green fluorescent protein (GFP), or the presence of donor genetic markers, such as the Y-chromosome, in many non-hematopoietic tissues [3-10]. Findings include the transdifferentiation of bone marrow cells into muscle cells [11], cardiac muscle [9], hepatocytes [12], astrocytes [13], and neurons [14-16]. Likewise, transplantation of cells derived from other tissues like brain, skin, muscle and fat, has given rise to different lineages distinct from their tissue of origin [6, 17-19].

The transdifferentiation of bone marrow cells was initially described at pathologic level to explore the mechanism and causes of many diseases of bone metabolism. It has long been argued that the abnormal direction of differentiation of bone marrow cells such as osteoblast and adipocyte will definitely have distinct impact on the process of bone formation [20]. Moreover, the pathologic switch of bone and fat in bone marrow also cause large controversy on the detailed mechanism which would mislead the normal status of cell to the wrong way.

Osteoporosis is a well-known disease that results from the unbalance of bone formation and bone absorption. Many researches considered

osteoporosis as a faultiness of BMMSCs committing to the adipocyte lineage because of their inability to differentiate into other cell lineages, such as osteoblasts. Moreover, in recent years, the transdifferentiation of bone and fat is the new insight of the increasingly bone marrow fat in elderly or menopause crowd. Some researchers previously suggest BMMSCs as a potent candidate to differentiate to different lineages regarding tissue engineering and bone remolding. It has been argued that bone-related cells might not be able to maintain their terminal differentiation state under physiological conditions and then dedifferentiated to a plasticity level and finally transdifferentiate to other lineages, which might lead to diseases like osteoporosis. Moreover, whether cells outside the bone marrow such as adipocyte derived stem cells (ADSCs) have the potential to transdifferentiate into bone lineage still arises large controversy.

In this review we focus on not only the progress and mechanism of transdifferentiation of bone and fat related to bone metabolism, but also the clinical prospect in the area of transdifferentiation. Moreover, it is of significance to understand bone and fat transdifferentiation on bone metabolism because there still have doubts concerning the reported findings of transdifferentiation, and it is clinically meaningful that transdifferentiation would benefit the same individual suffering from bone metabolism diseases like osteoporosis in cell replacement therapy allowing generation of transdifferentiated cells.

The aim is to provide a critical review of the reported findings, supplemented by our own preliminary results, and to present alternative explanations for functional effects attributed to bone and fat transdifferentiation. Finally, recommendations are presented to facilitate evaluation of this magic process.

Transdifferentiation of bone and fat

Osteoblasts and adipocytes are originated from common mesenchymal progenitor cells, and the differentiation of the two lineages is under the modulation of several transcription factors [21]. It has long been regarded that BMMSC-derived osteoblast has the potential to transdifferentiate directly to adipocyte lineage under proper conditions [22]. Furthermore, fully differentiated adipocytes presented in bone have been reported to undergo osteogenic switch modulated by proper transcriptional factors [23].

Previous studies showed that terminally differentiated adipocytes would be unable to proliferate or undergo dedifferentiation or mitosis [24, 25]. Meanwhile, non-terminally differentiated adipocytes maintain their ability to proliferate and have the potency to further differentiation [26].

Concerning to the adipo-osteogenic transdifferentiation, in particular, a relatively pure group of adipocytes from bone marrow cells seems to be capable of proliferate vigorously and subsequently undergo osteogenesis, which needs an intermediate step to induce morphological change into preadipocytes [27]. Another research indicated that subcutaneous preadipocytes have the capacity to differentiate into osteoblasts [28]. These findings showed that although lipid-filled mature adipocytes are not directly differentiated to osteoblasts, adipocytes are converted to mature osteoblasts through a preadipocytic stage during which the proliferation potential enhanced [29]. Bellows and coworkers [30] showed that 1, 25-dihydroxyvitamin D3 stimulates adipogenesis in cultures of fetal rat calvarial cells, while some studies have demonstrated the essential effect of glucocorticoids of low concentration in osteogenesis in mouse, rat, rabbit, and human adipocyte populations [31-35].

Based on these findings presented above, Triffitt et al. [36] further demonstrated that the transdifferentiation potential of osteogenic and adipocytic cells in the human bone marrow is proved by the progeny of cloned single adipocytes forming the osteogenic cultures. Nevertheless, we remain largely unknown as to what extent of adipocytes can differentiate meanwhile remain the ability to proliferate vigorously and subsequently differentiate in an osteogenic direction. Therefore, to date, research on the transdifferentiation capacity of adipocytes, which is essential for the understanding of bone turnover in metabolic diseases, has been limited.

On the other hand, the research of osteo-adipocytic transdifferentiation has also been widely conducted in recent years. Nuttall and cowork-

ers have shown that cells derived from explants of adult human trabecular bone have the capacity of adipogenesis when cultured in the presence of 3-isobutyl-1-methylxanthine (IBMX) and dexamethasone [23]. In these studies, further evidence of bone and fat transdifferentiation are reported, whereby a mature osteoblast will undergo adipogenesis because of the transdifferentiation. These previous studies clearly confirm that adipocytic and osteogenic cells share a common precursor in bone marrow and indicate an inverse relationship between adipogenesis and osteogenesis, although, single-cell analysis is required to confirm the lineage potential of an osteogenic/adipocyte precursor. By far, cloned single adipocytes were discovered to have the capacity of dedifferentiation into fibroblast-like cells, and subsequently change into two morphologically distinct cell types, osteoblasts and adipocytes, in certain culture systems. These findings validated the transdifferentiation potential between the osteogenic and adipocytic cells in human bone marrow stromal cell cultures, and proved that the osteogenic and adipocytic cells share a common multipotential precursor [27].

To further elucidate the detailed mechanism of bone and fat transdifferentiation. Our preliminary data indicated that BMMSC-derived osteoblasts and pre-osteoblast cell line MC3T3-E1 cells can undergo adipocytic transdifferentiation under the control of estrogen by canonical Wnt signaling pathway. We showed that the adipocyte transdifferentiated from osteoblast mainly express highly white adipocyte markers and relatively lower brown adipocyte markers, indicating that transdifferentiation-derived adipocyte are mainly the white adipocytes and have no difference with BMMSC-derived adipocytes. We demonstrated that fully differentiated osteoblasts still have the ability to transdifferentiate to adipocytic lineage but partially lose the ability when inhibited by estrogen, indicating that Wnt signaling pathway modulated by estrogen on osteo-adipocytic transdifferentiation could be a new explanation for clinic therapy of estrogen.

Therefore, transdifferentiation between bone and fat are available at certain conditions and require the involvement of dedifferentiation, proliferation and differentiation of BMMSCs. What's more, the direct switch of bone and fat in bone marrow require several complicated factors to be controlled and further efforts should be made to demonstrate this partially-understood phenomenon.

Cell signal of bone and fat transdifferentiation on bone metabolism

The molecular events associated with the transdifferentiation of osteoblasts and adipocytes are still largely unknown. Schilling et al. [37] found that transdifferentiated cells with normally differentiated cells showed amounts of reproducibly regulated genes for both, adipocytic and osteogenic transdifferentiation by microarray analyses. Several signaling pathways like Wnt, Runx2, Integrin and PPARγ signaling displayed clearly differential expression patterns of transdifferentiation. Most of genes modulated during osteogenic transdifferentiation showed elevated expression whereas the majority of genes regulated during adipocytic transdifferentiation were declined. This indicates that the osteogenic transdifferentiation of differentiated adipocytes is mainly achieved indirectly by down-regulation of gene products which would capable of inhibiting adipocytic transdifferentiation. In contrast, osteo-adipocytic transdifferentiation seems to require several active genes involved in lipid metabolism. Furthermore, Schilling et al. also demonstrated that adipocytic transdifferentiation starts 3 h after beginning with transcription-associated events, whereas after 24 h morphogenesis-, cytoskeleton-, and extracellular matrix-related genes are activated in both transdifferentiation directions. Additionally 24 h after initiation of osteogenic transdifferentiation, the accumulation of cell cycle- and chromosome-associated genes indicates a distinct variation of the cellular state. Accordingly, numbers of studies [30, 38, 39] have defined the conditions that are required in marrow stromal cultures for adipocytic transdifferentiation. The thiazolidinediones, which is a new class of synthetic antidiabetic compounds, binds to the peroxisome proliferator-activated receptor (PPARγ) and can induce adipogenesis in vitro, and in vivo have provided new approaches to understanding the mechanisms involved in bone and fat transdifferentiation [40]. Therefore, MSCs or pre-osteoblasts that have lost the potential to repress adipocytic switch could also be connected to the age- and disease-related gain of adipose

tissue in bone marrow and the concomitant loss of bone mass if some pathologic conditions activate some gene signaling to undergo osteo-adipocytic transdifferentiation [41-48].

As a whole, adipocytic transdifferentiation is driven by an intricate and well-orchestrated signaling cascade. It involves regulated changes in the expression and/or activity of several key transcription factors, most notably PPARγ and several members of the CCAAT/enhancer-binding family of proteins (C/EBPs) [49]. Also, Backesjo et al. [50] provided evidence for the essential effect of the inhibition of adipogenesis. They showed that the adipocytic conversion of murine MSCs was lessened by the activation of the nuclear NAD-dependent protein deacetylase sirtuin 1 under osteogenic incubation conditions.

What's more, it is recently reported that overexpression of Runx2 in adipose-derived stem cells stimulates the induction of osteoblastic differentiation which is accompanied by detectable levels of mineralized nodules and reduced *PPARγ* mRNA [51]. Differentiation into mature osteoblasts with increased Runx2 mRNA may also be induced by a preadipocytic cell line originated from mouse subcutaneous fat tissues [52]. These findings suggest that Runx2 is a key factor in the transdifferentiation of adipocytes into functional osteoblasts.

Takahashi et al. [21] used a retroviral gene-delivery system to examine the effect of Runx2 on the osteogenic transdifferentiation potential of 3T3-L1 cells. He found that osteogenesis was synergistically increased when introducing Runx2-overexpressing 3T3-L1 cells with the expression vector which encodes a full-length mitogen-activated protein kinase phosphatase-1 (MKP-1) gene. Thus, glucocorticoid-dependent osteogenesis involving Runx2 activity was regulated [53]. These findings proved that Runx2 and MKP-1 may play an essential role in the adipo-osteogenic transdifferentiation in bone marrow.

What's more, Skillington et al. showed that adipogenesis of 3T3-F442 preadipocytes is suppressed by over-expression of BMP receptors. Likewise, over-expression of BMP receptors also stimulates osteogenic transdifferentiation in response to retinoic acid, which results in the deposition of numerous mineralized nodules [54].

In recent years, it is found that the transcription of some components of integrin signaling is regulated during bone and fat transdifferentiation, amongst them villin 2 (ezrin; VIL2) and IGFBP1 obtained high bioinformatic scores. VIL2 is a membrane-cytoskeletal linking protein [55]. It regulates cell–cell and cell-matrix adhesions [56]. IGFBP1 binds to the a5b1 integrin receptor and also affects focal adhesion kinase phosphorylation [57]. During osteogenic transdifferentiation of the majority of these genes indicated changes by up-regulation in the actin cytoskeleton which is organized by integrins [58].

Moreover, genes involved in canonical Wnt signaling pathway or affected by it were observed in the transdifferentiation studies, particularly on tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin; TNFRSF11B), secreted frizzled-related protein 2 (SFRP2), cysteine-rich angiogenic inducer 61 (CYR61), angiopoietin-like 4 (ANGPTL4). Canonical Wnt signaling has previously been reported to promote osteogenic [59] while suppress adipocytic differentiation [60, 61]. Interestingly, consistent with our preliminary data, canonical Wnt signaling pathway was highly involved in osteo-adipocytic transdifferentiation and acted downstream of estrogen, indicating that Wnt family plays an essential role in bone and fat transdifferentiation. Further evidence should be proved in detail about the mechanism of bone and fat switch in human bone marrow.

Altogether, molecules and pathways involved in the process of bone and fat transdifferentiation set the stage for further functional studies to uncover remedy. It would be mandatory to initiate or prevent the lineage switch of osteoblasts and adipocytes in bone marrow. Moreover, scientific and targeting modulation of those signaling pathway provides us bright future of the therapy of transdifferentiation on bone metabolism.

Future and prospective therapy of transdifferentiation on bone metabolism

An increasing amount of experimental findings showed that bone marrow adipogenesis causes bone loss which is associated with aging, chronic drug treatment, and pathogenic disorders such as diabetes and osteoporosis [62-65]. However, studies on the underlying mecha-

nisms are only beginning to emerge. Transdifferentiation of osteo-adipogenic switch is probably a contributing factor. Therefore, to provide promising, novel pharmacologic targets, it is necessary to identify and characterize signaling pathways that influence or determine the phenotypic fate of transdifferentiated cells. Considering the limitations of current therapies for disorders of bone loss such as osteoporosis [66], the area of transdifferentiation is an attractive prospect. Moreover, it is significant to conduct further research and to emphasize fundamental problems of target tissue selectivity before the full realization of the potential of these approaches.

One potential advantage of the use of transdifferentiated cells for cell replacement therapy, if possible, would be that cells could be isolated directly from the potential recipient, transdifferentiated and grafted as an autologous cell graft without subsequent risks of immune reactions and need for immunosuppressive treatment and associated risks of side effects [67]. Although studies of transplantation of BMMSCs in animal models of osteopenia disorders have been reported many years ago, including osteoporosis, the specific mechanisms are still largely unknown. Here comes a question: if it is possible that the transplantation of BMMSCs to treat osteopenia disorders be replaced by other lineage-derived transdifferentiated cells such as ADSCs-derived osteoblasts or marrow adipocyte-derived osteoblasts and thus ignore the immunoreactions exist for so long time, providing unique and essential clinic vision for the remedy of bone-related diseases. Moreover, whether there exist some drugs that we can take advantage of to directly change the increasing bone marrow adipocytes to bone related cells like osteoblasts and chondrocytes to provide attractive remedy for clinical bone metabolic diseases.

Therefore, detailed investigation is necessary for the development of bone and fat transdifferentiation to solve fundamental issues currently limiting their widespread use.

Concluding remarks

By reviewing, it is clear that several determine issues need further clarification and examination before bone and fat transdifferentiation *in vivo* can be scientifically proven and accepted. It is likely to therapeutically apply such transdifferentiated cells. Specifically, it needs to answer whether transdifferentiation of bone and fat is biologically possible, i.e. whether it is a fact or an artifact. This will require general scientific acceptance of a set of criteria, which should contain the proof of stable (ideally long-term) expression of the "end point" biomarkers and cell characteristics. Meanwhile, it is necessary to define the exclusion of cell fusion interpreted as transdifferentiation, the exclusion of expression of the chosen "end point" cellular biomarkers by the cell sources before potential transdifferentiation, the exclusion of cellular leakage and uptake by other cells of cell tracking markers, such as transgene fluorescent proteins and confirmation *in vivo* of transdifferentiation results obtained *in vitro* [1].

Identification of all these factors will not only shed light on fundamental mechanisms modulating development but also provide tools to manipulate the transdifferentiation potential of bone and fat for cell-based approaches in the area of bone metabolism. Understanding what mechanisms and how pathways mediating the transdifferentiation between osteoblasts and adipocytes *in vivo* are modulated should be of relevance to the development of therapeutic control of bone loss in osteoporosis.

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Disclosure of conflict of interest

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