

# Transdifferentiation: why and how?

Macarena Perán<sup>1\*,†</sup>, Juan Antonio Marchal<sup>†,‡</sup>, Fernando Rodríguez-Serrano<sup>†,‡</sup>, Pablo Álvarez<sup>‡</sup> and Antonia Aránega<sup>†,‡</sup>

\* Department of Health Science, University of Jaén, 23071 Jaén, Spain

† Biopathology and Regenerative Medicine Institute (IBIMER), 18100 Granada, Spain

‡ Department of Human Anatomy and Embryology, University of Granada, 18012 Granada, Spain

## Abstract

Cell therapy is based on the replacement of damaged cells in order to restore injured tissues. The first consideration is that an abundant source of cells is needed; second, these cells should be immunologically compatible with the guest and third, there should be no real threat of these cells undergoing malignant transformation in the future. Given these requirements, already differentiated adult cells or adult stem cells obtained from the body of the patient appear to be the ideal candidates to meet all of these demands. The utilization of somatic cells also avoids numerous ethical and political drawbacks and concerns. Transdifferentiation is the phenomenon by which an adult differentiated cell switches to another differentiated cell. This paper reviews the importance of transdifferentiation, discussing the cells that are suitable for this process and the methods currently employed to induce the change in cell type.

Keywords: adult differentiated cell; cell therapy; stem cell; transdifferentiation

## 1. The relevance of transdifferentiation

The key to embryonic development is the acquisition of a differentiated identity. Two cells fuse into a single cell zygote, creating a multicellular organism. Processes in early development direct cells to differentiate and become specialized cells that will interact with one another to form specific tissues that then associate in all of the organs that form a complete organism. The acquisition and maintenance of a final cell differentiation state is crucial, and differentiation should proceed in a single direction, with no possibility of turning around or back. However, is this what really happens? Not really, since it has been demonstrated that already differentiated cells can change to another type of differentiated cell by a phenomenon known as transdifferentiation (Okada, 1991; Eguchi and Kodama, 1993; Slack and Tosh, 2001).

How is differentiation possible? The answer is that every cell of the organism has a complete and identical set of genes, and the magic of early development is the orchestration by which cells express only a reduced number of genes that distinguish them from neighbouring cells. Hence, gene expression may change to develop different cell types, but the full complement of genes remains present in all cells, and an appropriate induction mechanism could reverse the expression of specific genes, inducing differentiation and a switch to another cell type. In fact, a complete organism has been cloned from adult cells, demonstrating that somatic cells retain the potential to become any cell of the organism (Wilmut et al., 1997).

The aim of the emerging field of regenerative medicine is to provide the body with tools for self-repair and self-regeneration. Different disciplines are combining efforts in the search for new approaches to healing impaired body functions by repairing, replacing or regenerating cells, tissues or organs. The possibility

that a stable adult differentiated cell can transdifferentiate opens the door to novel therapeutic strategies against multiple diseases caused by the loss of indispensable, irreparable and specific cells, including diabetes, liver failure and neurodegenerative disorders. One cell therapy approach is to stimulate adult cells to transdifferentiate into neighbouring dysfunctional cells. The study of transdifferentiation is not only clinically relevant but also increases our knowledge on the factors that induce cells to differentiate, improving our understanding of normal developmental biology. The direction of differentiation in stem cells requires the identification of genes ('master genes') that are expressed in one cell type but not another and are responsible for the formation of different tissues (Burke and Tosh, 2005).

The present review focuses on advances in adult cell conversion, on the cells that are suitable for transdifferentiation and on methods proposed for directing cell differentiation.

## 2. Spontaneous transdifferentiation

It is logical to think that the natural occurrence of transdifferentiation implies a major dysfunction, since the body is programmed to maintain differentiated cells in their post. The inclusion of one type of differentiated cell into the environment of another type of differentiated cell occurs in diseases known clinically as metaplasias, which predispose the individual to develop tumours. In Barret's metaplasia, for instance, the stratified squamous epithelium that normally lines the lower part of the oesophagus, transdifferentiates into an intestinal-like tissue, with an increased risk of tumourigenesis (Wild and Hardie, 2003). Pathological transdifferentiation appears to result from a pathological stimulus. Thus, a fibrotic stimulus causes HSCs (hepatic stellate cells) to transdifferentiate from a quiescent phenotype to a collagen-producing myofibroblast-like phenotype and to proliferate,

<sup>1</sup>To whom correspondence should be addressed (email mperan@ugr.es).

**Abbreviations:** ADSCs, adipose tissue-derived stem cells; LIF, leukaemia inhibitor factor; MSCs, mesenchymal stem cells.

thereby producing liver fibrosis (Adachi et al., 2007). Furthermore, hypoxia, via myocardin regulation, is thought to induce transdifferentiation of endothelial cells into smooth muscle-like cells (Zhu et al., 2006), which can develop into a severe disease such as PAH (pulmonary arterial hypertension), characterized by increased pulmonary vascular resistance due to vasoconstriction and structural remodelling of pulmonary arterioles, with a thickening of medial vessel walls (Cheever, 2005).

On the other hand, animal studies have demonstrated that a naturally occurring transdifferentiation may be a defence mechanism, with evidence that adult differentiated cells can be redifferentiated into a damaged cell type for the repair of injured tissues. In fish and developing chick embryos, retinal pigment epithelial cells can transdifferentiate to sensory cells after damage of sensory receptor cells (Stroeva and Mitashov, 1983; Mitashov, 1997). In mammals, an example of spontaneous transdifferentiation as a defence against injury was reported by Park et al. (2006). In an extensive study of triple transgenic mice, they demonstrated that lung epithelial repair is driven by transdifferentiation of ciliated epithelial cells; after up-regulation of certain transcription factors critical for the embryonic differentiation of the lung, these cells are able to redifferentiate into cuboidal and then columnar cell types, contributing to restore the bronchiolar epithelium.

### 3. Which cells are indicated?

Various cells have been proposed as the best candidates for transdifferentiation. Some groups have focused on processes involving the direct differentiation of adult stem cells (metaplasia), while others have researched the interconversion of adult differentiated cells (transdifferentiation). These terms are not always correctly employed in the literature. Transdifferentiation is the switch between one differentiated cell type to another, while metaplasia should be used to refer to stem cell conversion (Slack and Tosh, 2001), but numerous authors describe the differentiation of adult stem cells as transdifferentiation (Parker and Katz, 2006; Perán et al., 2010a). We review here the transdifferentiation of adult differentiated cells and the use of adult stem cells in differentiation induction studies.

#### 3.1. Adult differentiated cells

Transdifferentiation experiments have been performed *in vivo* and *in vitro* in various systems. The transdifferentiation of adult differentiated cells is greatly facilitated when related cell types are selected, since cells that share a common embryonic origin are more likely to interconvert. In neighbouring tissues that differ in only a few transcription factors, up-regulation of the expression of the corresponding genes has proven sufficient to change cell type characteristics (Li et al., 2005).

Owing to their cell lineage proximity, the transdifferentiation of pancreas to liver cells and liver to pancreas cells has been studied, representing a well-documented example of cell interconversion (Burke et al., 2007). Numerous studies have supported the potential of pancreatic duct, acinar and endocrine cells to transdifferentiate

into hepatocytes, and rat pancreatic cell line and mouse fetal pancreatic tissue have been shown to express hepatic characteristics (Shen et al., 2000). Moreover, hepatocytes have been detected in islets of Langerhans of transgenic mice (Krakowski et al., 1999), and pancreatic acinar cells have also been transdifferentiated into hepatocytes (Lardon and Bouwens, 2005).

Other studies have shown that transdifferentiation of mature hepatocytes to pancreas is also possible. Numerous authors have reported the conversion of liver cells into pancreatic-like cells, which can even produce insulin (Ferber et al., 2000; Yang et al., 2002; Horb et al., 2003; Zalzman et al., 2003; Perán et al., 2010b).

Furthermore, endothelial cells can be isolated from the umbilical vein and used to regenerate myocardium after their transdifferentiation (Condorelli et al., 2001). In the lung, human fetal alveolar type II cells have been transdifferentiated into type I-like cells with increased epithelial cell barrier function (Foster et al., 2007).

#### 3.2. Adult stem cells

Adult (postnatal) stem cells persist throughout life, with the mission to repair or replace cells in response to natural cell turnover in certain tissues. There has been considerable research interest in their use in cell therapy, since various adult stem cells can be obtained from the patient with relative ease and cultured and manipulated *in vitro*. MSCs (mesenchymal stem cells) that derive from the embryonic mesenchyme are found in the stromal fraction of the bone marrow and adipose tissue, among other tissues (Musina et al., 2005). Human bone marrow-derived stem cells are multipotent cells that can differentiate into osteoblasts and adipocytes (Prockop, 1997; Pittenger et al., 1999; Verfaillie, 2002; Barry and Murphy, 2004). Although adult stem cells are committed to a developmental programme, they can switch into a cell type of a different lineage by genetic reprogramming (Song and Tuan, 2004). This 'stem cell plasticity' is exemplified by the ability of MSCs to differentiate into cells that are not typical mesenchymal derivatives (Krabbe et al., 2005) and, by their ability, with specific cues, to differentiate into neurons (Kondo et al., 2005). A recent study showed that bone marrow stromal cells can transdifferentiate into Schwann cells and myelinate axons, demonstrating their potential therapeutic value (Keilhoff et al., 2006).

Adipose tissue is a rich source of MSCs and provides an abundant and accessible supply of adult stem cells, designated ADSCs (adipose tissue-derived stem cells). ADSCs can be isolated from human lipoaspirates with minimal risk or discomfort for the patient and then differentiated into osteogenic, adipogenic, neurogenic, myogenic or chondrogenic lineages (Safford et al., 2002; Zuk et al., 2002; Miranville et al., 2004; Planat-Benard et al., 2004; Romanov et al., 2005). Our group recently demonstrated that adult cardiomyocytes from human donors retain their capacity to induce the cardiomyocyte differentiation of MSCs. Human adult stem cells obtained from lipoaspirates were transiently permeabilized and exposed to intracellular extracts obtained from adult human heart tissue, subsequently observing phenotypic and molecular modifications of ADSCs, with the expression of specific markers and cardiomyocyte-related genes indicating entry of treated cells into cardiomyogenic differentiation. Our findings showed that adult heart tissue can direct the transdifferentiation of human MSCs towards cardiomyocytes (Perán et al., 2010a).

## 4. Methods of transdifferentiation

We review here the methods used for transdifferentiation, focussing on the switch between adult tissue cells and on the direction of adult stem cell differentiation.

### 4.1. Defined culture medium

There is evidence that transdifferentiation can be induced by the addition of specific molecules from a wide range of families. Some researchers have added chemicals, some have used proteins (e.g. growth factors) and others have used synthetic molecules, such as the glucocorticoid dexamethasone. Thus, Tosh's group used dexamethasone to transdifferentiate a pancreatic cell line to a hepatic-like cell type, visualizing the posttreatment induction of up to eight liver-specific markers by immunolabelling with specific antibodies (Shen et al., 2003). The authors concluded that transdifferentiation involves a molecular pathway by which dexamethasone activates glucocorticoid receptors responsible for a cascade effect that ends in the activation of liver differentiation target genes (Shen et al., 2003).

Differentiation media have also been employed to transdifferentiate liver cells to pancreatic-like cells, growing liver cells (hepatic oval cells) in culture media supplemented with LIF (leukaemia inhibitor factor). After removing LIF from the medium and adding a high concentration (23 nM) of glucose, these cells transdifferentiated into various glucagon- and insulin-producing pancreatic cell types that proved able to reverse hyperglycaemia in streptozotocin-induced diabetes (Yang et al., 2002). Furthermore, with the use of a defined culture medium, pancreatic islet cells transdifferentiated to exocrine pancreatic cells and undifferentiated cells that can be considered pancreatic precursor (stem) cells; multilabel immunohistochemical and immunoelectron microscopy studies of the islets at different days of culture, using islet cell markers, revealed that endocrine cells gradually transdifferentiated to ductal, acinar and intermediary cells (Schmied et al., 2001).

Defined media have also been used to differentiate adult stem cells, finding chondrogenic differentiation of MSCs when cultured in the presence of dexamethasone, ascorbic acid phosphate and supplements (bovine insulin, transferrin, selenous acid, linoleic acid and BSA) (Sottile et al., 2002). In addition, the growth factor TGF- $\beta$ 1 appears to determine the transdifferentiation of MSCs to chondrogenic-like cells, which produce acid mucopolysaccharides, glycosaminoglycans and proteoglycans (Okamoto et al., 2002; Suva et al., 2004). In fact, BMPs (bone morphogenetic proteins) of the TGF- $\beta$ 1 superfamily have been shown to enhance bone formation and cartilage differentiation in cell culture systems (Hiraki et al., 1991; Katagiri et al., 1994; Hogan, 1996). The medium used to induce the osteogenic differentiation of MSCs *in vitro* consisted of dexamethasone, ascorbic acid-2-phosphate and beta-glycerophosphate (Okamoto et al., 2002). Osteogenic differentiation has been demonstrated by analysis of mineralized deposits (Sottile et al., 2002), by measurement of alkaline phosphatase activity (Yoshimura et al., 2007) and by RT-PCR study of the expression of specific osteogenic genes (Friedman et al., 2006; Lin et al., 2006).

For adipogenic differentiation, confluent cell cultures are treated with standard medium supplemented with dexamethasone, isobutylmethylxanthine and insulin. The presence of mature adipocytes is assessed under the microscope after Oil Red-O staining of the cultures (Sottile et al., 2002).

The differentiation of MSCs along osteogenic, adipogenic and chondrogenic lineages is being investigated using defined culture media in order to establish the potential plasticity of isolated mesenchymal cells (Koch et al., 2007; Liu et al., 2007; Stender et al., 2007).

Chemicals have also been used to direct MSC differentiation, for example, by incubating cells in serum-free medium containing 6  $\mu$ M 5-azacytidine for 24 h. Treated MSCs showed a cardiomyocyte-like morphology and were found to express cardiac differentiation markers and specific genes in the RT-PCR analyses (Kadivar et al., 2006; Rodríguez-Serrano et al., 2010).

### 4.2. Co-culture

In embryonic development, the cell microenvironment is critical for organogenesis (Tsai et al., 2002). The co-culture approach is based on the idea that one cell type can influence another via extracellular signal molecules and direct cell–cell contact (Hackney et al., 2002), with the microenvironment playing a crucial role in cell differentiation. In trials on the regeneration of infarcted myocardium with adult stem cells, it was reported that the differentiation of implanted cells might be influenced by exogenous factors from the tissue microenvironment (Wang et al., 2000; Collins et al., 2007; Giordano et al., 2007). However, other authors have postulated that cell fusion is responsible for the 'myocardialization' of the cells (Oh et al., 2003; Nygren et al., 2004).

Co-culture systems have been used to test the hypothesis that adult cells can transdifferentiate into myocytes in response to signals from neighbouring myocytes, using human endothelial progenitor cells, skeletal muscle-derived cells and bone marrow cells, among others (Badorff et al., 2003; Fukuhara et al., 2003; Iijima et al., 2003). Two different cell types are grown together in these systems, with some variations in the number of chambers and in the separation between cell types. In double-chamber systems, the cell types are separated by a membrane (0.4–0.3  $\mu$ m pore size) to avoid direct cell–cell interaction; this technique has been used to determine whether soluble chemical factors released from one cell type, and able to pass through the membrane, are sufficient to induce transdifferentiation of the other cell type. In direct co-culture experiments, the two cell types are labelled with distinct fluorescent tracers (e.g., DAPI, DIL or specific antibody) and then placed in the same dish or flask with no physical separation between them. This culture system was found to induce transdifferentiation by Yoon et al. (2005), who reported that direct cell–cell contacts rather than soluble factors underlie the transdifferentiation of MSCs into cardiomyocytes. Iijima et al., (2003) studied three different methods (monoculture, co-culture and double-chamber system) and also concluded that cell–cell contact is necessary for the transdifferentiation of skeletal muscle-derived cells into cardiomyocytes. These results strongly suggest that soluble factors alone are not sufficient to induce differentiation of MSCs and that physical contact between cardiomyocytes and MSCs is required,

as also shown by Wang et al. (2006). Other concurrent data have revealed the importance of Jagged1 protein-activated Notch signalling in the differentiation of MSCs into cardiomyocytes in co-culture systems (Li et al., 2006).

Hence, direct cell–cell contact between two cell types appears sufficient to induce transdifferentiation. Further research is required to establish whether cell fusion is exclusively responsible or whether physical stretch, receptor–ligand binding or the integrin adhesion molecule contribute to this transdifferentiation, and to identify the genes or transcription signals that regulate the process. Koyanagi et al. (2005) speculated that transdifferentiation in co-culture systems results from nanotubular communication that allows the transport and exchange of intracellular proteins, molecules and even organelles between the cell types, resembling a transient cell fusion. Accordingly, cells may influence each other via direct physical contact, creating a common intracellular space.

### 4.3. Transfection of transcription factors

'Master switch' genes are genes that direct undifferentiated cells towards a specific differentiated state. Thus, the ability to interconvert between liver and pancreas reflects the close developmental relationship between these tissues and supports the theory that transdifferentiation is driven by one or two master switch genes that differentiate the tissues in development (Burke et al., 2007). Well-documented examples of these genes include MyoD, believed to be crucial for myogenesis, and several genes involved in pancreatic formation, such as *Pdx1* (pancreatic–duodenal homeobox), *Ngn3* (neurogenin3), *Hes1* (hair cell enhancer-of-split-1) and *PTF1-p48* (pancreas transcription factor-1a/p48) genes. The transdifferentiation of fibroblasts into adipocyte-like cells has been achieved via the ectopic expression of the adipogenic transcription factors C/EBP (chicken CCAAT/enhancer binding protein) alpha, PPAR (peroxisome proliferator-activated receptor) gamma and SREBP-1 (sterol regulatory element-binding protein-1) (Liu et al., 2010). Furthermore, Vierbuchen et al. (2010) directly converted embryonic and postnatal rat fibroblasts into neurons by using a combination of three neural lineage-specific transcription factors: *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*.

In tissue engineering, MSCs can be genetically modified to express transcription factors or growth factors that induce differentiation along a desired lineage (Carlberg et al., 2001; Gelse et al., 2003; Katayama et al., 2004). The identification of master switch genes will be invaluable for directing stem cell differentiation towards liver, pancreatic, intestinal, neuronal or other phenotypes.

### 4.4. Cellular extract

The overexpression of a single transcription factor may not be enough to change the fate of a cell, and it is likely that multiple factors, cofactors and auxiliary molecules are also involved. Evidently, a technique that passes the complete genetic machinery from one cell type to another should produce a change in cell fate. The laboratory of Professor Collas developed a 'cellular extract method' for introducing the intracellular component of cells into

cells of a different type by incubating purified somatic nuclei, or reversibly permeabilized somatic cells, from one cell type with a nuclear and cytoplasmic extract derived from a different somatic ('target') cell type. This extract is believed to provide nuclear regulatory components that mediate alterations in the gene expression profile of the target genome. Following exposure to the extract, the cells are resealed in calcium-containing culture (Håkkelien et al., 2002; Landsverk et al., 2002). By this means, human fibroblasts have been successfully reprogrammed into T-like cells, using the cytoplasmic extract of human T cells (Håkkelien et al., 2002) and into beta-like cells (even able to produce insulin), using the extract of a rat insulin-producing  $\beta$  cell line (Håkkelien et al., 2004). In another experiment, human ADSCs produced cardiomyocyte proteins after incubation with rat cardiomyocyte extracts (Gaustad et al., 2004). The success of these transdifferentiation strategies was demonstrated by phase-contrast microscopy observations of morphological changes, by RT-PCR findings on donor cell-specific genes and by immunological evidence of alterations in protein expression (Håkkelien et al., 2002; Landsverk et al., 2002; Gaustad et al., 2004).

Our group recently reported that exposure of human ADSCs to a cellular extract obtained from adult human atrial biopsies induces the expression of cardiogenic markers (Perán et al., 2010a). We have also demonstrated the transdifferentiation of human hepatoma cells (HepG2 cells) to insulin-expressing cells by exposing HepG2 cells to an extract of RIN (rat insulinoma cells) (Perán et al., 2010b).

### 4.5. Nanotopography can direct transdifferentiation

Researchers experienced in cell culture are accustomed to the marvellous adaptive capacity of cells. We are familiar with the fact that they can be removed from their tissue organization and grown in a totally different artificial environment. Live cells in culture can be harvested by dramatic enzyme treatments, centrifuged and reseeded in a wide variety of different supports and remain alive. Some cells can be grown in confluence or even neglected in poor medium and still survive. This extraordinary survival ability of the cells should not be underestimated.

The extreme plasticity of cells has been used to transdifferentiate human MSCs into neuronal-like cells. Yim et al. (2007) showed that nanotopography can induce neuronal differentiation, growing human bone marrow-derived stem cells on nanogratings at least one order of magnitude smaller than the cell body; they literally stretched the cells and forced them to acquire a neuronal morphology, observing that the cytoskeleton and nuclei of the cells were significantly aligned and elongated along the direction of the grating axis. These morphological changes were accompanied by the up-regulation of neuronal markers such as Tuj1, MAP2 and GFAP.

## 5. Conclusions

A major health challenge is posed in developing countries by diseases that involve postmitotic tissues, whose cells have very low or no proliferative capacity.

The aim of regenerative medicine is to regenerate tissues with no initial capacity for regeneration, and there has been increasing scientific interest in the use of cell therapy for this purpose. The phenomenon of transdifferentiation, by which differentiated cells can change fate and become another differentiated cell type, may serve as a mechanism to obtain the abundant number of cells required for transplantation. One advantage of this approach is that damaged cells are replaced with cells from the same individual, minimizing the risk of immune attack.

The numerous candidate diseases for cell therapy include cardiac injury, degenerative cartilage diseases, diabetes mellitus and even neuronal dysfunction, which are all characterized by the loss of functional cells.

The initially promising results obtained with animal models encouraged researchers to carry out clinical trials. However, the benefits of cell implantation therapy in patients have been controversial. The outcomes of clinical trials have mandated a return to the laboratory and cell culture assays to address the problems that arise in humans. There are new relevant factors to be considered, including the role of cytokines and the microenvironment. Some authors have even questioned the value of introducing cells at all, if the injection of cytokines is able to activate the adult stem cell population that appears to lie dormant in every tissue.

Transdifferentiation can be used to direct the reprogramming of one cell type to another and may represent a useful tool in the emerging discipline of cell therapy. We have reviewed here some feasible transdifferentiation approaches with proven ability to reprogramme cells. However, considerable work is still needed to develop a definitive method for the long-lasting differentiation of cell types with therapeutic value.

## References

- Adachi M, Osawa Y, Uchinami H, Kitamura T, Accili D, Brenner DA. The forkhead transcription factor FoxO1 regulates proliferation and transdifferentiation of hepatic stellate cells. *Gastroenterology* 2007;132:1434–46.
- Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A et al. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation* 2003;107:1024–32.
- Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol* 2004;36:568–84.
- Burke Z, Tosh D. Therapeutic potential of transdifferentiated cells. *Clin Sci* 2005;108:309–21.
- Burke ZD, Shen CN, Ralphs KL, Tosh D. Characterization of liver function in transdifferentiated hepatocytes. *J Cell Physiol* 2006;206:147–59.
- Burke ZD, Thowfeequ S, Peran M, Tosh D. Stem cells in the adult pancreas and liver. *Biochem J* 2007;404(2):169–78.
- Carlberg AL, Pucci B, Rallapalli R, Tuan RS, Hall DJ. Efficient chondrogenic differentiation of mesenchymal cells in micromass culture by retroviral gene transfer of BMP-2. *Differentiation* 2001;67:128–38.
- Cheever KH. An overview of pulmonary arterial hypertension: risks, pathogenesis, clinical manifestations, and management. *J Cardiovasc Nurs* 2005;20:108–16;117–8.
- Collins SD, Baffour R, Waksman R. Cell therapy in myocardial infarction. *Cardiovasc Revasc Med* 2007;8:43–51.
- Condorelli G, Borello U, De Angelis L, Latronico M, Sirabella D, Coletta M et al. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. *Proc Natl Acad Sci USA* 2001;98:10733–8.
- Eguchi G, Kodama R. Transdifferentiation. *Curr Opin Cell Biol* 1993;5:1023–8.
- Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin induced hyperglycemia. *Nat Med* 2000;6:568–72.
- Foster CD, Varghese LS, Skalina RB, Gonzalez LW, Guttentag SH. *In vitro* transdifferentiation of human fetal type II cells toward a type I-like cell. *Pediatr Res* 2007;61:404–9.
- Friedman MS, Long MW, Hankenson KD. Osteogenic differentiation of human mesenchymal stem cells is regulated by bone morphogenetic protein-6. *J Cell Biochem* 2006;98:538–54.
- Fukuhara S, Tomita S, Yamashiro S, Morisaki T, Yutani C, Kitamura S et al. Direct cell–cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage *in vitro*. *J Thorac Cardiovasc Surg* 2003;125:1470–80.
- Gaustad KG, Boquest AC, Anderson B E, Gerdes AM, Collas P. Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. *Biochem Biophys Res Commun* 2004;314:420–7.
- Gelse K, von der MK, Aigner T, Park J, Schneider H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. *Arthritis Rheum* 2003;48 430–41.
- Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol* 2007;211:27–35.
- Guan K, Hasenfuss G. Do stem cells in the heart truly differentiate into cardiomyocytes? *J Mol Cell Cardiol* 2007;43:377–87.
- Hackney JA, Charbord P, Brunk BP, Stoeckert CJ, Lemischka IR, Moore KA. A molecular profile of a hematopoietic stem cell niche. *Proc Natl Acad Sci USA* 2002;99:13061–6.
- Häkkelien AM, Gaustad KG, Collas P. Transient alteration of cell fate using a nuclear and cytoplasmic extract of an insulinoma cell line. *Biochem Biophys Res Commun* 2004;316:834–41.
- Häkkelien AM, Landsverk HB, Robl JM, Skälhegg BS, Collas P. Reprogramming fibroblasts to express T-cell functions using cell extracts. *Nat Biotechnol* 2002;20:460–6.
- Hiraki Y, Inoue H, Shigeno C, Sanma Y, Bentz H, Rosen DM et al. Bone morphogenetic proteins (BMP-2 and BMP-3) promote growth and expression of differentiated phenotype of rabbit chondrocytes and osteoblastic MC3T3-E1 cells *in vitro*. *J Bone Min Res* 1991;6:1371–85.
- Hogan BLM. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev* 1996;10:1580–94.
- Horb ME, Shen CN, Tosh D, Slack JMW. Experimental conversion of liver to pancreas. *Curr Biol* 2003;13:105–15.
- Iijima Y, Nagai T, Mizukami M, Matsuura K, Ogura T, Wada H et al. Beating is necessary for transdifferentiation of skeletal muscle-derived cells into cardiomyocytes. *FASEB J* 2003;17:1361–3.
- Kadivar M, Khatami S, Mortazavi Y, Shokrgozar MA, Taghikhani M, Soleimani M. *In vitro* cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. *Biochem Biophys Res Commun* 2006;340:639–47.
- Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T et al. Bone morphogenetic protein-2 converts the differentiation pathway of C212 myoblasts into the osteoblast lineage. *J Cell Sci* 1994;127:1755–66.
- Katayama R, Wakitani S, Tsumaki N, Morita Y, Matsushita I, Gejo R et al. Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. *Rheumatology (Oxford)* 2004;43:980–5.
- Keilhoff G, Gohl A, Langnäse K, Fansa H, Wolfa G. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. *Eur J Cell Biol* 2006;85:11–24.
- Koch TG, Heerkens T, Thomsen PD, Betts DH. Isolation of mesenchymal stem cells from equine umbilical cord blood. *BMC Biotechnol* 2007;7:26.
- Kondo T, Johnson SA, Yoder MC, Romand R, Hashino E. Sonic hedgehog and retinoic acid synergistically promote sensory fate specification from bone marrow-derived pluripotent stem cells. *Proc Natl Acad Sci USA* 2005;102:4789–94.
- Koyanagi M, Brandes RP, Haendeler J, Zeiher AM, Dimmeler S. Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes? *Circ Res* 2005;96:1039–41.

- Krabbe C, Zimmer J, Meyer M. Neural transdifferentiation of mesenchymal stem cells—a critical review. *APMIS* 2005;113:831–44.
- Krakowski ML, Kritzik MR, Jones EM, Krahl T, Lee J, Arnush M et al. Pancreatic expression of keratinocyte growth factor leads to differentiation of islet hepatocytes and proliferation of duct cells. *Am J Pathol* 1999;154:683–91.
- Landsverk HB, Håkelién AM, Küntziger T, Robl JM, Skålhegg BS, Collas P. Reprogrammed gene expression in a somatic cell-free extract. *EMBO Rep* 2002;3:384–9.
- Lardon J, Bouwens L. Metaplasia in the pancreas. *Differentiation* 2005;73: 278–86.
- Li H, Yu B, Zhang Y, Pan Z, Xu W, Li H. Jagged1 protein enhances the differentiation of mesenchymal stem cells into cardiomyocytes. *Biochem Biophys Res Commun* 2006;341:320–5.
- Li WC, Yu WY, Quilan JM, Burke ZD, Tosh D. The molecular basis of transdifferentiation. *J Cell Mol Med* 2005;9:569–82.
- Lin Y, Liu L, Li Z, Qiao J, Wu L, Tang W et al. Pluripotency potential of human adipose-derived stem cells marked with exogenous green fluorescent protein. *Mol Cell Biochem* 2006;291:1–10.
- Liu TM, Martina M, Hutmacher DW, Hui JH, Lee EH, Lim B. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. *Stem Cells* 2007;25:750–60.
- Liu S, Wang Y, Wang L, Wang N, Li Y, Li H. Transdifferentiation of fibroblasts into adipocyte-like cells by chicken adipogenic transcription factors. *Comp Biochem Physiol A Mol Integr Physiol* 2010;156(4):502–8.
- Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumie A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation* 2004;110:349–55.
- Mitashov VI. Retinal regeneration in amphibians. *Int J Dev Biol* 1997;41:893–905.
- Musina RA, Bekchanova ES, Sukhikh GT. Comparison of mesenchymal stem cells obtained from different human tissues. *Bull Exp Biol Med* 2005;139:504–9.
- Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 2004;10:494–501.
- Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA* 2003;100:12313–8.
- Okada TS. *Transdifferentiation: flexibility in cell differentiation*. Clarendon Press; 1991.
- Okamoto T, Aoyama T, Nakayama T, Nakamata T, Hosaka T, Nishijo K et al. Clonal heterogeneity in differentiation potential of immortalized human mesenchymal stem cells. *Biochem Biophys Res Commun* 2002;295:354–61.
- Park KS, Wells JM, Zorn AM, Wert SE, Laubach VE, Fernandez LG et al. Transdifferentiation of ciliated cells during repair of the respiratory epithelium. *Am J Respir Cell Mol Biol* 2006;34:151–7.
- Parker AM, Katz AJ. Adipose-derived stem cells for the regeneration of damaged tissues. *Expert Opin Biol Ther* 2006;6:567–578.
- Perán M, Marchal JA, López E, Jiménez-Navarro M, Boulaiz H, Rodríguez-Serrano F et al. Human cardiac tissue induces transdifferentiation of adult stem cells towards cardiomyocytes. *Cytotherapy* 2010a;12:332–337.
- Perán M, Sanchez A, Marchal JA, López E, Alvarez P, Boulaiz H, et al. Ultrastructural and molecular analyses of insulin-producing cells induced from human hepatoma cells. *Cytotherapy* 2010b; doi:10.3109/14653249.2010.501791.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
- Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation* 2004;109:656–63.
- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276:71–4.
- Rodríguez-Serrano F, Alvarez P, Caba O, Picón M, Marchal JA, Perán M et al. Promotion of human adipose-derived stem cell proliferation mediated by exogenous nucleosides. *Cell Biol Int* 2010;34(9):917–24.
- Romanov YA, Darevskaya AN, Merzlikina NV, Buravkova LB. Mesenchymal stem cells from human bone marrow and adipose tissue: isolation, characterization, and differentiation potentialities. *Bull Exp Biol Med* 2005;140:138–43.
- Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM et al. Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun* 2002;294:371–9.
- Schmid BM, Ulrich A, Matsuzaki H, Ding X, Ricordi C, Weide L et al. Transdifferentiation of human islet cells in a long-term culture. *Pancreas* 2001;23:157–71.
- Shen CN, Seckl JR, Slack JM, Tosh D. Glucocorticoids suppress beta-cell development and induce hepatic metaplasia in embryonic pancreas. *Biochem J* 2003;375:41–50.
- Shen CN, Slack JMW, Tosh D. Molecular basis of transdifferentiation of pancreas to liver. *Nat Cell Biol* 2000;2:879–87.
- Slack JMW, Tosh D. Transdifferentiation and metaplasia – switching cell types. *Curr Opin Genet Dev* 2001;11:581–6.
- Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. *FASEB J* 2004;18:980–2.
- Sottile V, Halleux C, Bassilana F, Keller H, Seuwen K. Stem cell characteristics of human trabecular bone-derived cells. *Bone* 2002;30:699–704.
- Stender S, Murphy M, O'Brien T, Stengaard C, Ulrich-Vinther M, Soballe K et al. Adeno-associated viral vector transduction of human mesenchymal stem cells. *Eur Cell Mater* 2007;13:93–9.
- Stroeva OG, Mitashov VI. Retinal pigment epithelium: proliferation and differentiation during development and regeneration. *Int Rev Cytol* 1983;83:221–93.
- Suva D, Garavaglia G, Menetrey J, Chapuis B, Hoffmeyer P, Bernheim L et al. Non-hematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. *J Cell Physiol* 2004;198:110–8.
- Tsai RY, Kittappa R, McKay RD. Plasticity, niches, and the use of stem cells. *Dev Cell* 2002;2:707–12.
- Verfaillie CM. Adult stem cells: assessing the case for pluripotency. *Trends Cell Biol* 2002;12:502–8.
- Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 2010;463:1035–41.
- Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. *J Thorac Cardiovasc Surg* 2000;120:999–1006.
- Wang T, Xu Z, Jiang W, Ma A. Cell-to-cell contact induces mesenchymal stem cell to differentiate into cardiomyocyte and smooth muscle cell. *International Journal of Cardiology* 2006;109:74–81.
- Wild CP, Hardie LJ. Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat Rev Cancer* 2003;3:676–84.
- Wilmot I, Schnieke AE, McWhir J, Kind AJ, Campbell K H. Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997;385:810–3.
- Yang L, Li S, Hatch H, Ahrens K, Cornelius JG, Petersen BE et al. In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci USA* 2002;99:8078–83.
- Yim EK, Pang SW, Leong KW. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp Cell Res* 2007;313:1820–9.
- Yoon J, Shim WJ, Ro YM, Lim DS. Transdifferentiation of mesenchymal stem cells into cardiomyocytes by direct cell-to-cell contact with neonatal cardiomyocyte but not adult cardiomyocytes. *Ann Hematol* 2005;84:715–21.
- Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue and muscle. *Cell Tissue Res* 2007;327:449–62.

Zalzman M, Gupta S, Giri RK, Berkovich I, Sappal BS, Karnieli O et al. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *Proc Natl Acad Sci USA* 2003;100:7253–8.

Zhu P, Huang L, Ge X, Yan F, Wu R, Ao Q. Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle-like

cells regulated by myocardin involved in hypoxia-induced pulmonary vascular remodelling. *Int J Exp Path* 2006;87:463–74.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13:4279–95.

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