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Review

Exploring the cell signalling in hepatocyte differentiation

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ABSTRACT

The liver is the second largest organ in the human body and is responsible for several functions that directly contribute to homeostasis. Hepatocytes are the main parenchymal liver cells that regulate multiple biochemical and metabolic functions and the synthesis of substances important to the body. Mesenchymal stem cells (MSCs) are a group of stem cells derived from the mesoderm, which can be obtained from various tissues. Under certain conditions, MSCs can differentiate into several cell types, including hepatocytes. Post-transcriptional regulations of liver development signalling and hepatocyte differentiation have been demonstrated. At the post-transcriptional level, microRNAs have emerged as precursors for determining cell fate during differentiation. MicroRNAs (miRNAs) are small non-coding RNAs involved in the post-transcriptional regulation of gene expression. They can determine the stem cell fate by repressing the translation of target mRNAs. In this review, we outline signalling pathways involved in stem cell differentiation to hepatocytes and its interplay with liver development. Hepatic differentiation models in two-dimensional and three-dimensional cultures used to analyse signalling mechanisms will be described. We also highlight the possible miRNAs involved in this process and the transdifferentiation signalling mechanisms present in hepatocytes.

Contents

1. Introduction

In recent years, stem cells have generated great interest owing to their potential therapeutic uses. Under the influence of environmental factors, including extracellular matrix components, the factors secreted by cells or cell-cell interactions can promote the proliferation, migration, and differentiation into multiple cell types that compose the human body and/or replace the damaged cells from adult tissues [1].

Cell differentiation implies a sequence of orchestrated events that coordinate the conversion of stem cells and precursors to a particular specialised cell type and involves the loss of stem cell characteristics as well as the acquisition of specialised functions and specific markers [2]. At the post-transcriptional level, microRNAs have emerged as precursors in the control of proliferation and cell fate determination during

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cell differentiation [3]. MiRNAs are small non-coding RNAs involved in the post-transcriptional regulation. They can determine stem cell fate by repressing the translation of target mRNAs [4].

Currently, several studies have identified different miRNAs as mediators of stem cell differentiation into various cell types. Among them, miR-1 [5] and miR-24 [6] play an important role in myoblast and myocardial cell differentiation, and miR-9 [7] stands out during neural differentiation of bone marrow-derived MSCs. MiR-181 and miR-7641 play an important role in the differentiation of embryonic stem cells (ESCs) to endothelial cells [8,9]. In the adipogenic differentiation of MSCs derived from adipose tissue, miR-204, miR-211, miR-17-5p, miR-106a, miR-637, and miR-30e are highlighted due to their increased expression, whereas, during osteogenic differentiation, the expression of miR-21 and miR-22 is elevated in these stem cells [10]. However, few studies have characterised the expression of miRNAs during the differentiation of stem cells into hepatocytes.

Although stem cells differentiate into functional hepatocyte-like cells, a highly efficient method for the easy differentiation of stem cells into hepatocytes, without loss of viability and function, remains to be established. This difficulty is also encountered in primary hepatocyte culture, either during or after the transplantation of these cells [11,12]. Several factors influence cell differentiation and function, including physical cell-cell and cell-matrix interactions and spatial organization, and these properties are important in tissues that maintain functional parenchyma. Therefore, when working with primary cultures *in vitro*, the conditions are tailored to preserve cell characteristics, and the most suitable methods involve performing cultures in three-dimensional structures (3D) as scaffolds [12].

In this review, we collected comprehensive information available in the literature on stem cell differentiation into hepatocytes and suggested other possible factors that influence this process. Furthermore, we propose hepatic differentiation models in two- and three-dimensional cultures, describe the possible miRNAs involved in this process, and decipher the transdifferentiation mechanisms in hepatocytes.

2. Differentiation in liver development

Liver development mechanisms have been investigated in many animal and cell culture models. Many genes and molecular pathways that regulate embryonic liver development have been identified and are being manipulated to yield better result during *in vitro* culture. Intrinsic and extrinsic signals are responsible for both the mobile communication and interaction between cells, tissues and, organs and are capable of stimulating or inhibiting cellular processes.

Several cytokines and growth factors that are known to affect *in vitro* hepatocyte differentiation have been investigated in studies involving cultures and embryonic development of animals [13]. For instance, Hepatocyte Growth Factor (HGF) promotes the proliferation and migration of hepatoblasts as well as the activation of liver-specific genes. Oncostatin M (OSM) is responsible for hepatocyte maturation [14]. Epidermal growth factor (EGF) assists in hepatocyte growth and albumin helps in maintaining its expression [15]. Transforming Growth Factor (TGF) helps maintain the expression of liver-specific genes [16]. Basic Fibroblast Growth Factor (bFGF) induces liver development [17] and insulin assists hepatocyte maturation [18]. Insulin-like Growth Factor (IGF) induces the increased expression of Hepatocyte Nuclear Factor 1 alpha (HNF-1α) and Hepatocyte Nuclear Factor 4 alpha (HNF-4α). In addition, chemical compounds are also known to affect hepatocyte differentiation. For instance, dexamethasone (Dex) assists hepatocyte maturation [18], retinoic acid induces hepatocyte differentiation and maturation [19], sodium butyrate aids liver differentiation, demonstrating increased albumin expression [20], nicotinamide (NTA) assists hepatocyte proliferation [21], and noradrenaline and dimethylsulphoxide are used to accelerate hepatic differentiation [22] and increase the transcription of liver-specific genes [23].

Studies show that embryogenesis in mammals gives rise to endodermal epithelium of the gastrointestinal tract and the respiratory system, including the hepatocytes and biliary epithelium. The morphogenesis of these tissues begins with the expression of specific genes in a particular portion of the endoderm, causing specialised cells to proliferate and migrate from the endodermal tissue layer to form a sprout [24]. Nodal signalling is an important signal transduction pathway in differentiation and pattern formation during embryonic development and is triggered by TGF, which induces endoderm formation at high concentrations and mesoderm formation at low concentrations [24]. In addition, temporal gradients and spatial expression of FGF, Wnt (Wingless-type), bone morphogenetic protein (BMP), and retinoic acid that are secreted from the adjacent mesoderm are responsible for endoderm regionalization [24]. Hepatic bud formation begins with signals released from the cardiac mesoderm and the transverse septum that induce cell proliferation in the ventral mesoderm, which begins to invade the mesenchyme surrounding the transverse septa, as hepatoblasts are bipotential cells that later form the hepatocytes and cholangiocytes. Thus, the process of liver formation begins with the formation of the liver bud [24] (Fig. 1).

In addition, experiments have shown that the absence of FGF-1 and FGF-2 interferes with the induction of hepatic development [25]. Jung and colleagues used *in vitro* experiments to show that the cardiac mesoderm releases FGF 1, 2, and 8, culminating in hepatic bud formation from the gut endoderm [26]. Furthermore, the transverse threshold secretes BMP-2 and BMP-4, which induce the expression of specific genes in the liver cells of the ventral endoderm and their migration into the surrounding mesenchyme to form the hepatic bud [25,27]. In contrast, hepatocyte maturation is triggered and maintained by OSM along with Dex [14] (Fig. 1).

Although the signalling mechanisms involved still remain unknown, the information regarding mammalian development generated from

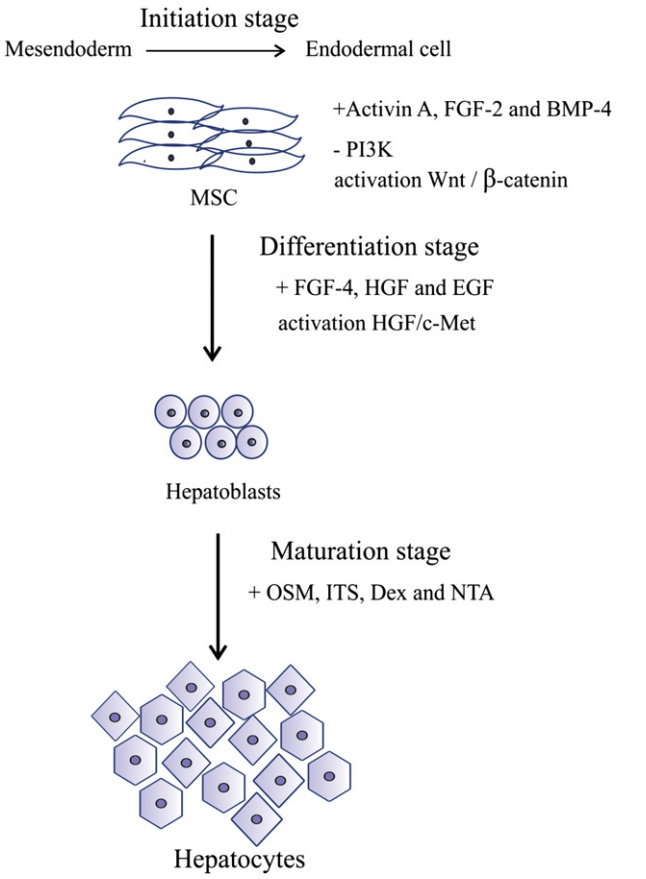

Fig. 1. *In vitro* differentiation consists of three stages: initiation, differentiation, and maturation. Please refer to the text.