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Reusing and composing models of cell fate regulation of human bone precursor cells

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ABSTRACT

In order to treat osteoporosis and other bone mass disorders it is necessary to understand the regulatory processes that control the cell fate decisions responsible for going from bone precursor cells to bone tissue. Many processes interact to regulate cell division, differentiation and apoptosis. There are models for these basic processes, but not for their interactions. In this work we use the theory of switched systems, reuse and composition of validated models to describe the cell fate decisions leading to bone and fat formation. We describe the differentiation of osteo-adipo progenitor cells by composing its model with differentiation stimuli. We use the activation of the Wnt pathway as stimulus to osteoblast lineage, including regulation of cell division and apoptosis. This model is our first step to simulate physiological responses *in silico* to treatments for bone mass disorders.

1. Introduction

A high percentage of the human population suffer diseases such as osteoporosis that affect: one third of women and one twelfth of men over 50 years old. The current treatments for increasing bone mass or reducing resorption have many limitations and side effects (Hoeppner et al., 2009). There is a strong opposition between bone and fat formation. Obesity reduces bone density and is inversely associated with bone formation in osteoporosis (Chen et al., 2010). There is a notorious decrease of the bone/fat formation ratio with the aging (Stenderup et al., 2003; Brockstedt et al., 1992). In this scenario, understanding regulatory signaling pathways that are relevant during control of bone formation (*e.g.* Wnt-mediated signaling) have emerged as critical components to treat in the future this and other bone disorders (Chen et al., 2010; Krishnan et al., 2006; Shahnazari et al., 2008; Kubota et al., 2009; Issack et al., 2008; Hoeppner et al., 2009). Moreover, it has become necessary to define their contribution within the regulatory processes that control the cell fate decisions responsible for going from bone precursor cells to bone tissue.

In this work, we analyze the process of bone and fat formation at a cellular level. We describe the dynamics of osteoblasts (bone cells), adipocytes (fat cells) and precursors. In such system, many processes interact in order to control the cell division, to regulate apoptosis, and to decide which cell lineages are produced. As proved by Chen et al. (2010), osteoblasts and adipocytes share a common precursor derived from the bone marrow stromal cells. These precursor cells can differentiate into osteoblast or adipocyte lineages depending on regulation signals. The Wnt/β-catenin pathway constitutes a potential target for bone mass disorder treatments such as osteoporosis or to reduce adiposity or fracture risk (Issack et al., 2008; Hoeppner et al., 2009). Its activation promotes osteoblast differentiation, proliferation and mineralization, and blocks apoptosis and osteoclastogenesis (Krishnan et al., 2006). On the other hand, the activation of *PPARγ* (*peroxisome proliferator-activated receptor gamma*) provokes adipogenesis (Chen et al., 2010).

For an approach to this complex system we consider the paradigm of Systems Biology, in which the behaviors emerge from the interaction between different processes (Kitano, 2002). Answers such as a specific increase of osteoblast concentration are provoked by the combined action activating the Wnt/β-catenin pathway, repressing the expression of *PPARγ*, and repressing the stimuli to osteoblasts apoptosis. Despite the existing models for

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each individual process, models for cross-talks and functional interactions between them have not been developed yet. Based on reusing existing models of individual processes, and combining them, we look for describing the process of bone and fat formation to analyze it *in silico*. The development of an accurate combined model will allows us to analyze *in silico* the physiological responses to treatments of bone mass disorders based on the Wnt signaling pathway, and to explore the efficiency of new medical strategies before testing them in animal models. At the current phase, our model predicts expected qualitative behaviors: activation or repression of each cell lineage.

Motivated by the recent model proposed by Schittler et al. (2010) and the results of Chen et al. (2010), we defined the expression of *RUNX*2 (*runt-related transcription factor 2*) as associated with the osteogenic differentiation (Krishnan et al., 2006; Lian et al., 2003), while *PPARγ* (*peroxisome proliferator-activated receptor gamma*) as associated with adipogenesis (Chen et al., 2010). Both transcription factors are mutually exclusive and auto regulated. This inter-regulated system is modeled by our main osteo-adipo switch model. We describe the differentiation from osteo-adipo progenitor cells into osteoblasts and adipocytes by associating the main osteo-adipo switch model with a well-described model of the Wnt/β-catenin pathway (Kim et al., 2007) to stimulate the osteoblast lineage, and with a probabilistic model that describes the activation of the *PPARγ* pathway during stimulation of the adipocyte differentiation (Krishnan et al., 2006; Chen et al., 2010). To accomplish this, we consider stimuli coefficients of the main osteo-adipo switch model as functions of the pathways activation. Finally, we include one good established and validated model (Kim et al., 2006) that reflects how apoptosis is controlled. We call such a combined model the cell fate decisions model.

The paper is structured as follows: Section 2 describes the material and methods used here: the biological system, the use of Systems Biology to consider emerging behaviors, the reused models and the implementation using BioRica; Section 3 presents the theoretical elements considered here: Gene Regulatory Networks and Switched Systems, combination of models; Section 4 present our models: the osteo-adipo switch model that introduces the Wnt pathway as bone formation stimulus, and the combined model for describing cell fate decisions for osteo-adipo differentiation; Section 5 shows and compares the simulation results of the combined models; Section 6 concludes and discusses the scopes and future improvements of our work.

2. Materials and methods

2.1. The biological systems: from progenitor cells to osteoblasts and adipocytes

In this paper we describe the dynamics for formation of osteoblasts and adipocytes from a common precursor derived from the bone marrow stromal cells. We model this system by using the Systems Biology paradigm (Kitano, 2002, Section 2.3). The differentiation of precursor cells into osteoblast and adipocyte lineage depends on many regulation processes as we describe here.

In multi-cellular organisms, inter and intra-cellular processes control the metabolism (Greenwald, 1998; Bukauskas, 1991). The basic function of a cell can be explained by four essential processes: growth, division, differentiation and apoptosis (Alberts et al., 2002). These processes are responsible for going from a unique cell to several specialized cell lineages. The cell decision between self-renewal, differentiation and apoptosis defines the cell fate (Wagers et al., 2002).

The cell cycle is comprised of several events that lead to cell division itself (mitosis and cytokinesis). It is regulated by several proteins named *cyclins* and *cyclin dependent kinases* (Alberts et al., 2002). The process through which an undifferentiated cell acquires specialized functions is called differentiation. During this process the undifferentiated precursor cell, called progenitor, can differentiate into different specific lineages. Thus, hematopoietic (blood) cells differentiate from a common progenitor into red blood cells, to transport oxygen, or into white blood cells that have defensive functions in the organism (Huang et al., 2007). Osteoblasts (bone cells) and adipocytes (fat cells) share a common precursor derived from the bone marrow stromal cells (Chen et al., 2010).

The Wnt/β-catenin pathway plays an important role in the stimulation of bone formation (Krishnan et al., 2006). It promotes osteoblat differentiation, proliferation and mineralization, and blocks apoptosis and osteoclastogenesis. Consequently, it is fundamental during bone remodeling and repair, constituting a potential target for the treatment of bone mass disorders such as osteoporosis or to reduce adiposity or fracture risk (Issack et al., 2008; Hoeppner et al., 2009). As example, it has been shown that loss of function of *LRP4* and *LRP5* (Wnt receptors, Krishnan et al., 2006) is associated with osteoporosis (Kumar et al., 2011). The presence of some Wnt ligands activates the canonical Wnt pathway and induces the accumulation of β-catenin in the nucleus of the cell, which interacts with a *TCF/LEF* transcription factor to activate the expression of the so-called Wnt target genes (Hodar et al., 2010). Some proteins that stimulate bone formation, such as *RUNX2* considered here, are Wnt targets (Krishnan et al., 2006).

Apoptosis, programmed cell death, can occur during cell-cycle or during the differentiation. It is controlled by a diverse range of cell signals, which may originate after either intrinsic or extrinsic inducers. Intracellular apoptosis begins in response to a stress such as heat, radiation, nutrient deprivation, viral infection, or membrane damage (Hunziker et al., 2010). Extracellular lethal signals include toxins, hormones, growth factors, nitric oxide or cytokines. In the case of bone cells, it has been shown that *homocysteine* induces strongly apoptosis in bone precursors and osteoblasts via the mitochondria pathway (Kim et al., 2006). The mechanisms here considered are shown in Fig. 1(A).

2.2. Reused models

To describe cell differentiation we reuse the osteo-chondro switch model proposed by Schittler et al. (2010) (Fig. 2), but replacing chondrocyte lineage variable by adipocyte lineage (see Section 4.1). The model corresponds to a description by differential equations of a Gene Regulatory Network (see Section 3.1), implemented here. We validated our implementation by comparing our results on the osteo-chondro switch model with the published results. The parameters of this model were adjusted a posteriori to human behaviors according to the results by De Ugarte et al. (2003), Schmidmaier et al. (2006), Manolagas (2000) and Arner et al. (2010). More details in Section 4.4.

About the stimulus models, we considered models for human cells built *a priori*. To simulate the induction of differentiation, we combined this model with other specific models that describe an engagement to the osteoblast lineage (by activation of the Wnt/β catenin pathway) or to the adipocyte lineage (by activation of the PPAR/γ pathway). Hence, we call the *main osteo-adipo switch model* the differentiation model before specifying the stimulus models, and the *osteo-adipo switch model* the model that includes the specific inductions to osteoblast and adipocyte lineages. To simulate the activation of the Wnt/β-catenin pathway, we reuse the model by Kim et al. (2007) (specified in SBML) performing the analyses under both control conditions and considering the activation of the Wnt pathway for a period of 500–1000 min. On the other hand, the activation of the *PPARγ*-pathway is stochastically simulated.

With regard to apoptosis, we reuse the model proposed by Kim et al. (2006) that explains how *homocysteine* (*Hcy*) induces strongly the apoptosis of bone precursors and osteoblasts in a mitochondria-mediated manner. According to the results obtained in *HS-5* osteoblastic lineage and primary human bone marrow stromal cells (both lines from ATCC, Manasas, VA, USA), when the concentration of *Hcy* is 10 μM the apoptosis rates of osteoblasts and precursors are increased to 47% and 41% respectively. We implemented directly this effect as an external factor on the differentiation process (see Section 4.3).

We include as part of our combined model a component computing the period between cell divisions. Due to the complexity of the cell cycle of human cells, currently we consider this period as a parameter of the model. While minimal models such as Tyson (1991) and Goldbeter (1991) are valid for simple organisms like yeasts, for human colon carcinomas cells are considered models such as presented by Haberichter et al. (2007).

2.3. Our goal and the need for combining models

Our main goal of this work is to build a model capable of predicting bone or fat formation considering a scenario near *in vitro* or *in vivo* conditions. As we explained in the previous section, there are many factors affecting bone formation in human body. The differentiation process, responsible for producing osteoblasts and adipocytes from precursor cells, depends on multiple regulation mechanisms. So, for modeling the dynamics of such cell lineages it is necessary to consider the dynamics of those mechanisms.

It is incorrect to describe the osteoblast formation by considering just a model validated on specific conditions of regulation mechanisms here considered. The activation or inhibition of the Wnt signaling pathway, the *PPARγ* expression, the division of progenitor cells, and the apoptosis of progenitor or osteoblast cells, work as a whole to generate osteoblasts and adipocytes from precursor cells. By using the Systems Biology paradigm (Kitano, 2002), we consider each mechanism separately and define the interaction between their models. The bone formation is considered an emerging property of the interacting processes. We mathematically describe each control mechanism effect, such as the activation of the Wnt pathway, by considering a specific coefficient of the differentiation model to be controlled by such mechanism. Reusing regulation mechanism validated models assure their accuracy and the construction of good combined models focus on the accurate inclusion of interactions.