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## **ISOLATION AND CHARACTERIZATION OF VISCERAL- AND SUBCUTANEOUS ADIPOSE-DERIVED MESENCHYMAL STEM CELLS: PUTATIVE ROLE IN OBESITY AND METABOLIC**

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The traditional view of adipose tissue as a passive reservoir for energy storage is no longer valid. As early as 1987, adipose tissue was identified as a major site for metabolism of sex steroids (1) and production of adiponectin, an endocrine factor that is markedly down-regulated in rodent obesity (2). The subsequent identification and characterization of leptin in 1994 firmly established adipose tissue as an endocrine organ(3). Adipose tissue is now known to express and secrete a variety of bioactive peptides, known as adipokines, which act at both the local (autocrine/paracrine) and systemic (endocrine) level. In addition to these efferent signals, adipose tissue expresses numerous receptors that allow it to respond to afferent signals from traditional hormone systems as well as the central nervous system (CNS). The important endocrine function of adipose tissue is emphasized by the adverse metabolic consequences of both adipose tissue excess and deficiency. Adipose tissue excess or obesity, particularly in the visceral compartment, is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, and prothrombotic and proinflammatory states (4). The prevalence of obesity and these associated morbidities, known as the metabolic syndrome (MetS), has reached epidemic proportions (4). Interestingly, adipose tissue deficiency or lipodystrophy is also associated with features of the metabolic syndrome in both humans and rodents (5). Metabolic syndrome (MetS) is defined as a constellation of metabolic disorders in the same individual with frequency higher than it could be expected by chance. MetS is one of the most widespread diseases in the world. MetS is evident in almost half of the population of specific age groups in developed countries. The search for the genetic basis of complex pathological syndromes represents a challenge for experimental medicine and genetics. Indeed, the identification of single genes involved in such multifactorial diseases may be frustrating due to the intrinsic complexity of the processes involved and of their genetic determinants and because of the limitation of actual knowledge on gene functions and all the relative networks. Nevertheless, we believe that the investigation on crucial cellular processes, such as energetics, redox metabolism, mitochondria-nuclei crosstalk, which could be

primarily involved in the onset of different multifactorial diseases, might allow the identification of few triggering genetic determinants. (6;7). Obesity has a strong genetic predisposition, and results from an excess energy intake and/or too little energy expenditure. Obesity is in most, but not all, subjects, associated with marked changes in the secretory function of adipocytes and macrophages, together with chronic low-grade inflammation and an increased risk to develop insulin resistance, diabetes, and/or vascular disease (8). Macrophages are more prevalent in adipose tissue of obese subjects than in adipose tissue of lean subjects and the macrophage quantity correlates with measures of insulin resistance (9). Both macrophages and adipocytes are capable of accumulating lipids and secreting cytokines. Interestingly, the number of macrophages in adipose tissue is reduced after weight loss (10).

Many studies showed the existence of multipotent adult progenitor cells in various tissues MSC isolated from tissues other than BM, as periosteum (11), trabecular bone (12), synovium (13), skeletal muscle (14), lung (16), thymus, spleen, pancreas, kidney (17; 18), peripheral blood (19), umbilical cord blood (20) and placenta (21). In the past ten years, studies reported that human adipose tissue contains a population of multipotent stem cells (MSC) (15), isolated in large amounts from liposuction or biopsy, and expanded in culture. Stromal-vascular cells of adipose tissue are adipose precursor and their differentiation in vitro depends on specific steps: adipoblast (unipotential cells), commitment preadipose cell (preadipocyte), terminal differentiation immature adipose cell, and terminal differentiation mature adipose cell (adipocyte) (22) Visceral adipose tissue associated with Metabolic Syndrome is a chemotactic niche, whereby mesenchymal stem cells can home to and differentiate into adipocytes to perpetuate its tissue formation (23). Our aim was to detect the expression of leptin, adiponectin and TNF alpha in visceral and subcutaneous adipose tissue derived from obese (BMI >30) and normal weight (BMI <30) patients with and without metabolic syndrome (MetS) and to isolate adipose derived stem cell (ADSC) to analyze the expression pattern of embryonic stem cell markers in obesity and metabolic syndrome. Results suggest that subcutaneous adipose tissue is

characterized by an increased endocrine/pro inflammatory activity and this increment is more evident in obese patients with MetS. This results suggest that subcutaneous adipose tissue in abdominal district in presence of obesity related to metabolic syndrome could have an intensive cytokine production. ADSCs isolated from VAT and SAT biopsies from obese and normal weight patients express the same levels of MSCs surfaces marker CD105 and CD-90 and embryonic stem cell markers SSEA-4, and the expression level of CD90, CD-105 and SSEA-4 was 90%, 78% and 0,2% respectively. mRNA expression level of pluripotency-associated genes reveals that only Nanog is more expressed in ADSCs isolated from subcutaneous adipose tissue of obese patients than ADSCs isolated from visceral adipose tissue. Other pluripotency-associated genes as Sox2, Oct4, c-Kit, CD-90, CD-73, CD-105, ABCG2, have the same expression level. Recently, a research group evidenced an adipotaxis phenomenon in an animal model, where the MSCs of the Bone Marrow migrated attracted to the fat depot by TNF-Alfa (245). This mechanism supports the idea of MSCs abnormal migration in patients with metabolic syndromet. We hypothesize that subcutaneous adipose tissue in obese patients with MetS is characterized by an intensive cytokine production and this may be responsible for ADSCs migration towards subcutaneous adipose tissue and visceral adipose tissue.

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