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Systemic prostanoid pattern analysis during adipogenic differentiation of human bone marrow mesenchymal stem cells

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Prostaglandins (PGs) play a complex role in adipogenesis (1). Depends on the differentiation stages, adipogenesis can be either promoted or inhibited by particular PGs (2-4). For example, prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) appear to suppress adipogenesis of various pre-adipocytes, including 3T3-L1 cells whereas prostacyclin (PGI_2) acts as an adipogenic agonist. In addition, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is one of the most potent activating ligands for peroxisome proliferator-activated receptor gamma (PPARγ), the transcription factor playing a pivotal role in adipogenesis (1, 5, 6). Cyclooxygenase (COX) enzymes, COX-1 and COX-2, mediate the rate-determining step to synthesize prostaglandin H_2 (PGH_2) from arachidonic acid (4, 7). Subsequently, PGH_2 is converted into the specific PGs by various prostaglandin isomerases. Interestingly, a recent study showed that heterozygous

COX-2 knockout (KO) mice developed obese phenotype but not homozygous (8, 9). Therefore, these KO studies suggested that PGs may have a complex role in the regulation of adipogenesis.

Human mesenchymal stem cells (hMSCs) can differentiate into adipocytes (10-11). hMSCs are widely distributed in a variety of tissues and organs. In obesity, it has been suggested that hMSCs may be recruited to a new adipocyte pool by metabolic changes (11-13). However, the molecular and cellular mechanisms of the adipogenesis have been intensively studied using murine pre-adipocyte systems, such as 3T3-L1 cells. The hMSCs may be advantageous over murine pre-adipocyte systems to study the molecular aspects of obesity and related metabolic diseases. In murine pre-adipocytes, the adipogenic cocktail, including insulin, dexamethasone, isobutylmethyxanthine (IBMX) (IDX), is enough to convert virtually all pre-adipocytes into fully differentiated adipocytes (14). However, it has been known that over 90% of hMSCs in culture flasks remain undifferentiated by the addition of IDX (10, 11). For hMSCs, PPARγ agonists have been used to increase cell population with adipocyte phenotype (10-12).

Human bone marrow MSCs (hBM-MSCs) constitutively express both COX-1 and COX-2 and produce PGs in culture (15). PGs synthesized by hBM-MSCs may regulate their adipogenesis by an autocrine or paracrine manner (9). The transcriptional and translational changes of COX enzymes, prostaglandin isomerases, and prostaglandin receptors in the prostanoid pathway may provide

insights to understand paracrine and autocrine regulations by PGs in adipogenesis. COX enzymes and membrane receptors for PGs have been well characterized in murine 3T3-L1 and Ob1771 pre-adipocytes (4, 7, 16, 17). However, the mRNA expression patterns of both enzymes and PG receptors over adipogenesis are not fully studied in hBM-MSCs. Compared to the murine pre-adipocytes, the presence of PPARγ agonists is critical to promote the differentiation of hBM-MSCs (11). In addition, it has been known that PPARγ agonists themselves can modulate transcriptions of the COX genes in several cell culture systems (18, 19). Therefore, if it is possible to measure transcriptional changes of all components in the prostanoid pathway during adipogenesis, it may provide the systemic information to understand autocrine and paracrine circuits of PGs in the hBM-MSCs and the role of PGs in metabolic diseases as well.

In the present studies, we evaluated the transcriptional patterns of various genes in the prostanoid pathway during adipogenesis of hBM-MSCs. First, we measured PG levels in various cell culture conditions and found significant reduction of PGE_2 and PGD_2 synthesis from pro-adipogenic conditions. Moreover, among the conditions with the IDX cocktail during adipogenesis, the PGE_2 level was significantly lower in the presence of PPARγ agonists compared to those without PPARγ agonists. In spite of the down-regulation of PGE_2 and PGD_2 levels in culture, the quantitative real-time reverse transcription polymerase chain reaction (Q-RT-PCR) studies showed that the mRNA levels of both COX-1 and

COX-2 were significantly increased upon the stimulation of adipogenesis compared to that of the control. Interestingly, regardless of the existence of PPARγ agonists, there were no significant differences in the mRNA levels of COX-1 and COX-2 when the IDX cocktail existed in culture. Exogenously added PGE_2 inhibited adipogenesis of hBM-MSCs (IC_{50} ~1 nM), whereas PGD_2 had no significant effect up to 1 μM. These results implicated that the down-regulation of PGE_2 biosynthesis may explain the difference between the presence and absence of PPARγ agonists for inducing the adipogenesis of hBM-MSCs.

In order to understand why PGE_2 and PGD_2 levels were decreased in spite of the up-regulation of COX-1 and COX-2 mRNAs during adipogenesis, we measured the transcriptional changes of several prostaglandin isomerases for PGE_2 and PGD_2. Of three prostaglandin E synthases, prostaglandin E synthase 1 (PTGES1) transcription was gradually decreased as the number of cells with lipid droplets increased in hBM-MSC culture. In contrast, there was no significant change in the transcription of both PTGES2 and PTGES3. Out of two prostaglandin D isomerases, mRNA level of lipocalin-type prostaglandin D_2 synthase (PTGDS) was decreased during adipogenesis.

Next, we determined the transcriptional changes of prostaglandin EP receptors to analyze the autocrine and paracrine regulation through the prostanoid pathway during the adipogenesis of hBM-MSCs. We found that the mRNA levels of prostaglandin EP2 and EP3 receptor were up-regulated whereas the mRNA of

prostaglandin EP4 was down-regulated. Importantly, the transcription of EP3 receptor subtype was correlated to the level of adipocyte differentiation whereas there was no significant difference in the mRNA levels of EP2 receptor between pro-adipogenic conditions with and without PPARγ agonists. These results suggested that EP2 and EP3 receptors be regulated by different manners in terms of the autocrine/paracrine control by PGE_2 during adipogenesis.

With the same biological samples used in the Q-RT-PCR studies, we performed a whole genomic microarray study with Affymetrix GeneChip™ Human Genome U133A 2.0 arrays. The Q-RT-PCR studies on the prostanoid pathway were well correlated to the whole genomic microarray data. The verification of the prostanoid pathway in hBM-MSCs before performing whole genomic measurement may provide reliability to the profiling data of other metabolic pathways whose data are generated from an 'omics' scale like microarray.

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