D-2. Effects of bovine lactoferrin on the motile activity of fibroblasts(Abstracts of the International Symposium on Recent Advances in Animal Science(IS-RAAS),Joint meeting of 2^<nd> IS-AS and 3^<rd> IS-IFS)

D-1. The winding road to candidate genes for marbling

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Most adipocyte differentiation occurs in late prenatal and early postnatal development, however, the intramuscular fat is the final adipose tissue depot as remarkably seen in meat animals, after skeletal and muscle growth have begun to slow down. Wagyu (Japanese black breed) have the ability to produce a high degree of marbling in skeletal muscle and seemed to be appropriate for study of the genes related to the adipocyte differentiation. In order to exclude the complicated environmental effects *in vivo*, we have established a preadipocyte line (BIP cell) from Wagyu intramuscular tissue. Exponentially growing BIP cells exhibit a fibroblastic appearance. BIP cells have the ability to differentiate to mature adipocyte. The metabolisms in BIP cells are different to those in 3T3-L1 cells, a murine cell line. For example, BIP cells have been shown not to express GLUT-4, an insulin-responsive glucose transporter, during adipogenesis. In addition, acetate is the main precursor for fatty acid synthesis. For the purpose of isolation of candidate genes concerning with bovine adipocyte differentiation, we have constructed a subtraction cDNA library from BIP cells. A total of 621 clones were picked up and sequenced. Of these clone, 443 clones (71.3%) were identified as homologues of known genes and 124 clones (20.0%) showed homology with bovine EST clones. The remaining 54 clones (8.7%) did not show homology with reported sequences and were classified as novel genes. In this presentation, I will discuss some of isolated genes.

D-2. Effects of bovine lactoferrin on the motile activity of fibroblasts

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Fibroblasts plated on a type I collagen gel can reduce the size of the gel as the culture period increase. The extent of collagen gel contraction reflects the motile activity of the fibroblasts. In addition to this, the collagen gel contractile activity of the fibroblasts is considered as an *in vitro* model for the reorganization of the collagen matrix during wound healing process. It has been shown that the bovine milk and colostrum stimulated the wound healing activity of fibroblasts.

Bovine lactoferrin (bLf), an abundant protein in the bovine milk and colostrum, was found to enhance the collagen gel contractile activity of WI-38 human fibroblasts. Lactoferrin is known as an iron binding protein, however, the effects of bLf on the collagen gel contractile activity of WI-38 fibroblasts was independent of the iron ion. The fibroblast migration promoting activity of bLf was confirmed by in vitro wound healing assay. Treatment of the fibroblasts with bLf enhanced the myosin light chain (MLC) phosphorylation within 30 min. The activities of bLf for MLC phosphorylation and collagen gel contraction was inhibited by inhibitors of Rho-kinase (Y-27632) and myosin light chain kinase (ML-7). These results suggest that bLf promotes the motility of the fibroblasts by the mechanism to regulate MLC phosphorylation.