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学位論文審査の要旨

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学位論文題名

Pregnancy specific regulation of lysosomal cathepsins in bovine blood leukocytes

(ウシ末梢白血球におけるリソソームカテプシンの妊娠特異的応答機構に関する研究)

This thesis consists of 113 pages including 7 chapters, 23 figures, 1 table, 1 supplementary table and 1 reference paper.

In ruminants, interferon tau (IFNT) is an important pregnancy recognition signal secreted from the preimplantation embryo which secretion level peaks at day18-19 before starting implantation. IFNT-mediated expression of interferon stimulated genes (ISGs) in peripheral blood leukocytes (PBLs) can indicate pregnancy marker. Recently, activation of lysosomes and lysosomal cathepsins (CTSs) by type 1 IFN signal transduction pathway was observed in immune cells. This study was conducted to explore the dynamics of lysosomes and lysosomal CTSs in bovine leukocytes during early pregnancy as a potential novel marker.

1. Pregnancy specific response of lysosomal CTSs in bovine leukocytes

This study aimed to explore the IFNT-mediated lysosomal activation in PBLs during early pregnancy of cows. Multiparous Holstein Friesian cows were subjected to artificial insemination (AI). PBLs collected from peripheral bloods on d18 of pregnant and non-pregnant cows were separated and used for the measurement of lysosomal acidification, activities of CTS B and K, expression of *LAMP-1*, *-2* and *CTSB* and *K*. Lysosomal acidification, CTS B and K activities were significantly increased in the pregnant PBLs. Gene expression levels of *LAMP-1*, *-2* and *CTS B and K* were also significantly increased in the pregnant PBLs than non-pregnant cells. Immunodetection revealed a significant increase of CTSK and LAMP-1 in pregnant PBLs. These results showed the first observation of pregnancy-specific lysosomal activation in bovine PBLs.

2. Status of lysosomes and lysosomal CTSs in bovine PBMCs and PMNs during early pregnancy

This study aimed to investigate the cell specific difference of lysosomal activation in the separated peripheral blood monocytes (MCs) and granulocytes GCs). Blood samples were

collected on d0, 7, 14 and 18 after AI. The activities of CTSs B, K and L were increased significantly both in MCs and GCs in the progress of pregnancy. Expression levels of *CTSB* and *K* were significantly increased both in MCs and GCs in pregnant cows. Lysosomal activity significantly increased both in MCs and GCs in pregnant cows. Expression level of *LAMP-1*, -2 was increased significantly in d18 pregnant cows. Immuno detection revealed the increase of CTSB both in MCs and GCs in pregnant cows. The findings of this study suggest that pregnancy specific activation of lysosome and lysosomal CTSs occurs both in MCs and GCs.

3. Effect of IFNT on stimulation of the activity of lysosomes and lysosomal CTSs in bovine MCs and GCs

To elucidate the direct effect of IFNT secreted from conceptus for the activation of lysosomal functions, in vitro experiment with recombinant IFNT (rbIFNT) was performed. Addition of IFNT significantly increased the activities of CTSs B, K and L both in PBMCs and PMNs. Gene expression levels of *CTSB* and *K* were also increased significantly both in PBMCs and PMNs by IFNT stimulation. Lysosomal activity was increased significantly in PBMCs and PMNs. Immuno detection revealed the increase of CTSB both in PBMCs and PMNs. These results suggest that activation of lysosomal and CTSs activity is stimulated by IFNT both in PBMCs and PMNs.

4. Involvement of type 1 IFN signal transduction pathway on lysosomal function in bovine leukocytes

The pregnancy-specific lysosomal activation of PBLs is highly possible by IFNT via type I IFN signal transduction pathway. However, the mechanism is unclear. This study was conducted to investigate the type I IFN pathway mediated activation of lysosome and CTSs. Lysosomal activity, expression levels of *LAMP-1*, -2 as wells as activities of CTSB and *K* were significantly increased by IFNT to non-pregnant PBLs. In contrast, addition of AZD1480, a JAK inhibitor, significantly decreased the lysosomal acidity and CTSK activity, expression of *LAMP-1*, -2 and *CTSK* in the presence of IFNT. Immuno detection also showed a significant increase of CTSK and *LAMP-1* after IFNT stimulation and decreased significantly after AZD1480. These results suggest that lysosomal function in leukocytes is regulated by IFNT via a type I IFN mediated pathway.

It is concluded that the lysosomes and lysosomal CTSs activity, expressions and protein level could be responsive to IFNT during maternal-fetal recognition period of pregnancy in PBLs via type I IFN signal transduction pathway and also lysosome and lysosomal CTSs potentially be useful biomarkers for early pregnancy detection.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.