

Validation of a transcriptomics analysis using real-time qPCR: Deciphering embryo-maternal communication

S.E. Ulbrich¹, C. Klein², S. Bauersachs^{2,3}, R. Einspanier³, E. Wolf^{2,3}, H.H.D. Meyer¹

¹Physiology Weihenstephan, Technical University Munich, Freising, Germany

²Institute of Molecular Animal Breeding and Biotechnology, Ludwig-Maximilian-University Munich, Munich, Germany
³Laboratory for Functional Genome Analysis (LAFUGA), Ludwig-Maximilian-University Munich, Munich, Germany

⁴Institute of Veterinary Biochemistry, Free University of Berlin, Berlin, Germany



ulbrich@wzw.tum.de

Introduction

A successful implantation and early embryonic development requires complex signaling mechanisms between the embryo and the uterus. In an attempt to identify genes which are presumably involved in embryo-maternal communication and induced in the bovine endometrium by the signaling of the embryo, a transcriptomics approach was commenced. A combination of subtracted cDNA libraries and cDNA array hybridization was applied to compare the pregnant and non-pregnant endometrium. The present study aimed to verify the results of the array hybridizations using real-time RT-qPCR, a highly sensitive and reliable mRNA quantification technique.

Materials and Methods

Five monozygotic twin pairs (Simmentaler Fleckvieh) generated by embryo splitting were cycle synchronized. At day 7 after standing heat two *in vitro* produced embryos were transferred into the ipsilateral uterine horn of one twin of each pair (Fig.1). The corresponding twin received a sham-transfer without embryos as a control. At day 18 the animals were slaughtered. The uterine lumen was opened longitudinally. Intercaruncular endometrial tissue samples from defined uterine regions which had been in contact with an embryo were used and corresponding tissue in the non-gravid twin. A combination of subtracted cDNA libraries and cDNA array hybridization was applied [1] to identify differentially expressed genes in the gravid compared to the non-gravid uterus. To validate cDNA array hybridization data mRNA concentrations of nine selected genes were relatively quantified by real-time RT-qPCR (LightCycler®). The same RNA samples were used

Results and Discussion

The combination of SSH and microarray technique revealed 90 different up-regulated genes and mRNAs. A classification of the genes based on Gene Ontologies revealed further the up-regulation of genes important for cell adhesion, cell differentiation, and cell communication (Fig.2). For all eight transcripts, RT-PCR results were in good correlation with the results obtained by array hybridization. Due to technical reasons of the hybridization for broad differences the array tends to underestimate transcript differences [1]. Further comparative analyses will now follow in order to confirm the relevance of the results in a large number of individual samples as well as a in more detailed compartments of the uterus and

Conclusions

The differential gene expression obtained by the holistic transcriptomics approach was clearly validated and strengthened in more detail by qRT-PCR. A combination of both transcriptomic and candidate gene approach seems most promising to identify and study thoroughly mechanisms of embryo-maternal signalling.

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"Mechanisms of embryo-maternal communication" [1] Bauersachs et al. (2005) J. Mol. Endocrinol. 34:359-368

Fig.1: Overview of the experimental design

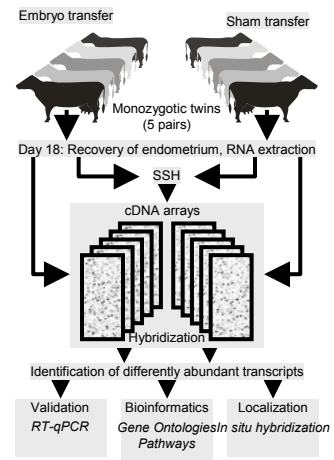


Fig.2: Classification of the identified genes into functional categories.

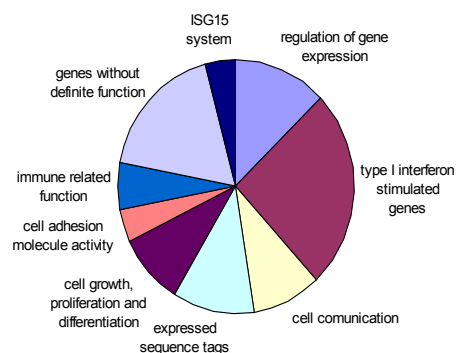


Fig.2: More than one third of the different up-regulated genes and mRNAs are known to be stimulated by type I interferons.

Fig.3: Validation of selected regulated genes by quantitative real-time RT-PCR

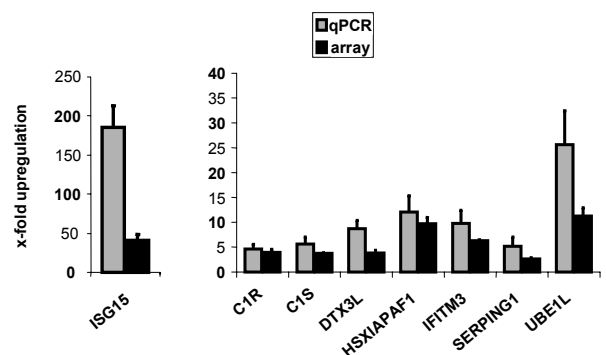


Fig. 3 Data are presented as x-fold upregulation of the gravid vs. the non-gravid twin uterus \pm SEM for both the qPCR and array approach. The most markedly upregulation was found for the interferon τ (IFN τ) stimulated gene 15 (ISG15).