Poster D-4

Discovering cell dynamics in pancreas fibrosis by means of microarray gene expression profiling

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Short Abstract: Comparing numbers of immediately versus belatedly regulated genes during activation of pancreatic stellate cells microarray gene expression profiling revealed the &quote;delay&quote; phenomenon. Cells pass an unexpected several days lag phase in stimulus response before they alter to fibrotic tissue. This aspect motivates for new anti-fibrotic therapeutic approaches in cancer research.

Long Abstract:

Keywords: oligonucleotide microarrays, gene expression analysis, cell mechanism, fibrosis, pancreas Summary: Using microarrays offers different familiar advantages for biomedical research including target identification, disease classification or characterization. Microarrays give a limited but comprehensive overview of gene activity in living cells. In certain cases, the differential gene expression analysis of transcripts even allows the discovery of cell dynamics. We present an application from pancreatic cancer research to show this unusual, but very effective usecase of microarrays. Confirming our gene expression profiling results from the transcriptome level by reverse transcription-polymerase chain reaction (RT-PCR) on the proteome level, the microarray approach provided insight into the so far unknown dynamics of molecular mechanisms involved in the activation process of pancreatic stellate cells (PSC). Clinical background: Pancreatic stellate cells are known to be crucially involved in the development of pancreas fibrosis, a characteristic feature of pancreatic cancer as well as of chronic pancreatitis. The development of anti-fibrotic therapy strategies requires the analysis of molecular mechanisms in PSC. The key event in the pathogenesis of fibrosis represents a transition process of quiescent into fully activated cells. PSC cultures from rat pancreas represent an accepted model for studies on cellular and molecular mechanisms of this activation process. While the differences between the quiescent and the activated PSC are well defined [1], the dynamics of the complex activation processes in PSC is poorly understood. So far an immediate activation process characterized by a continuous regulation of gene expression was assumed. Our data suggest that quiescent PSC in response to a stimulus pass a lag phase of several days before the activation process occurs. Material and Methods: Using microarray analysis we characterized gene expression profiles during PSC activation caused by in vitro cultivation on day 2 (state of quiescence), day 4 and 7 (intermediate activation stage), and day 14 (complete activation) after cell isolation. Four independent experiments each comprising three to four RNA samples of the respective day (14 arrays in total) have been performed with an adjacent quality control for replicated samples [2]. The array similarity of replicates was determined in relation to gene expression values (signals) in order to retain the two most similar arrays of each time point for differential expression analysis [3]. A gene with an at least +2/-2 fold change in its expression was defined to be up-/down regulated. In addition to

the signals themselves the respective detection calls (describing a transcript to be &quote;present&quote;, &quote;absent&quote; or &quote;marginal&quote; detected) were analyzed. These calls indicate the validity of signals. For a valid up regulation between two time points including both, a &quote;switch on&quote; of genes and an expression up regulation, two arbitrary calls in the initial day and a &quote;present&quote;-twin in the final day was required to include the gene into the analysis. Accordingly were a &quote;present&quote;-twin initially and arbitrary calls finally required for a valid down regulation. The subsequent gene expression profiling concerning all four time points showed genes with continuously in-/decreasing signals with a constant trend of regulation during the PSC activation, and also genes, which were primarily undergoing an activation delay during day 2 and day 4 with an afterwards instating expression regulation. Thus, two expression profile types were defined regarding signals of genes. A gene was assigned to a continuous regulation profile, if it was regulated between day 2 and day 4 and continuously in-/decreased thereafter (stepwise folds > 1). Genes indicating a delayed response profile are not required to be regulated in their gene expression between day 2 and day 4, but they should between day 2 and day 14. Incipient from day 4 they show a continuous in-/decreasing expression. Genes of interest that have been found in the microarray experiments to be differentially expressed during the transition of PSC were subjected to RT-PCR analysis. Results and discussion: In the present study, we show that the PSC activation process was associated with characteristic changes in the expression profiles of transcripts. 325 genes were differentially expressed in PSC starting from the state of quiescence up to a complete activation. 9 genes were continuously up regulated in contrast to 139 genes with a delayed response. Respectively, 46 genes showed a continuous profile of down regulation versus 131 genes with a delay in their repression. Our results suggest that PSC undergo a lag phase of several days before they respond to any activation stimulus. However, 83% of differentially expressed genes were regulated only after day 4 (intermediate activation stage) towards the final level of fully activated cells. In contrast, an immediate continious regulation could be detected in only a fifth of all regulated genes (17%). The delay phase in the beginning of the activation process may indicate an unstable state of cells concerning the direction of cell development. Closer meshed RT-PCR experiments suggest that in the following there is a &quote;point of no return&quote;. It was achieved between day 3 and day 5 after cell isolation. We suppose that initial processes of activation start between isolation and day 2 of culture, and they are completed on day 5 with a final irreversible cell state. In summary, the microarray gene expression profiling revealed by comparing the numbers of immediately and belatedly regulated genes a phenomenon that we referred to as delayed response to the activation stimulus in PSC. Even though the molecular mechanisms of PSC activation including the above discussed delay phase are of notable complexity, the understanding of this phenomenon is highly motivated, because it may provide the basis of novel anti-fibrotic therapeutic approaches. Acknowledgments: The study was supported with European Regional Development Funds EFRE 0400210/2004. 1. Sparmann G, Hohenadl C, Tornoe J, et al. Generation and characterization of immortalized rat pancreatic stellate cells. Am J Physiol Gastrointest Liver Physiol. 2004;287:211-219. 2. Glass A, Henning J, Karopka T, et al. Representation of individual gene expression in completely pooled mRNA samples. Biosci Biotechnol Biochem. 2005;69:1098-1103. 3. Bansemer S, Scheel T, Glass Ä, Karopka T: gEn0M - Software. In European Conference on Computational Biology 2. Paris, 2003:397-398.