Mining the transcriptome - methods and applications

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Abstract

Regulation of gene expression occupies a central role in the control of the flow of genetic information from genes to proteins. Regulatory events on multiple levels ensure that the majority of the genes are expressed under controlled circumstances to yield temporally controlled, cell and tissue-specific expression patterns. The combined set of expressed RNA transcripts constitutes the transcriptome of a cell, and can be analysed on a large-scale using both sequencing and microarray-based methods.

The objective of this work has been to develop tools for analysis of the transcriptomes (methods), and to gain new insights into several aspects of the stem cell transcriptome (applications). During recent years expectations of stem cells as a resource for treatment of various disorders have emerged. The successful use of endogenously stimulated or ex vivo expanded stem cells in the clinic requires an understanding of mechanisms controlling their proliferation and self-renewal.

This thesis describes the development of tools that facilitate analysis of minute amounts of stem cells, including RNA amplification methods and generation of a cDNA array enriched for genes expressed in neural stem cells. The results demonstrate that the proposed amplification method faithfully preserves the transcript expression pattern. An analysis of the feasibility of a neurosphere assay (in vitro model system for study of neural stem cells) clearly shows that the culturing induces changes that need to be taken into account in design of future comparative studies. An expressed sequence tag analysis of neural stem cells and their in vivo microenvironment is also presented, providing an unbiased large-scale screening of the neural stem cell transcriptome. In addition, molecular mechanisms underlying the control of stem cell self-renewal are investigated. One study identifies the proto-oncogene Trp53 (p53) as a negative regulator of neural stem cell self-renewal, while a second study identifies genes involved in the maintenance of the hematopoietic stem cell phenotype.

To facilitate future analysis of neural stem cells, all microarray data generated is publicly available through the ArrayExpress microarray data repository, and the expressed sequence tag data is available through the GenBank.

Key Words
transcriptome, gene expression profiling, EST, microarray, RNA amplification, stem cells, neurosphere