Network: Diseases Due to Environmental Factors

Project: From Pathway to Target - Pipeline to SME-driven Research

Dirk Seegert - CONARIS Research Institute AG - d.seegert@conaris.de

Introduction

Modern high through-put technologies such as biochip based expression screening, mutation detection by sequencing and/or real-time PCR, positional cloning and others have revealed a big number of new promissing disease targets in the past. However, in the future it will be necessary to develop further evaluation techniques, which allow a systematic functional characterization of these targets and which support the quick and economic identification of real drug (target) candidates.

Project Status

The CONARIS Research Institute AG started in 2000 with the extensive screening for disease associated genes and proteins in patients with inflammatory disorders of the intestinal tract. Since then a new technological platform was developed which enables the fast functional evaluation of unknown target candidates by the systematic screening of several different inflammatory and/or apoptotic parameters:

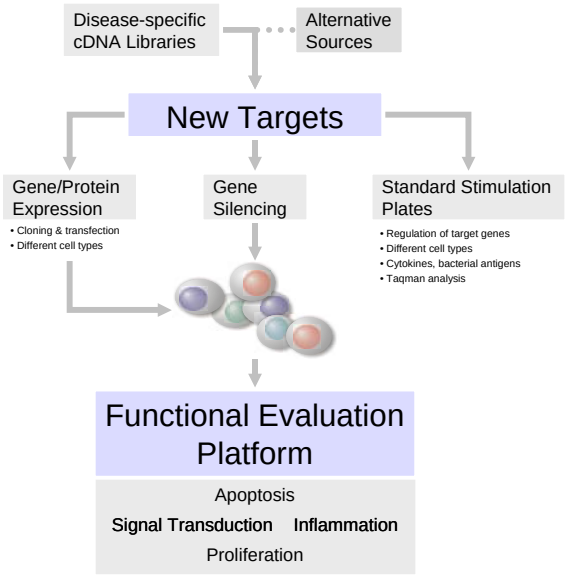

Fig 1: *Functional characterization of new targets by their biological influence on inflammatory/apoptotic parameters and mechanisms.*

From genes to clones

The first step of each evaluation project comprises the cloning of the gene of interest (GoI) into a suitable vector system (GATEWAY, Invitrogen), a sequence verification and the subsequent transfection of the resulting constructs into different cell types (T cells: Jurkat; Monocytes/macrophages: THP-1; epithelial cells: HEK293, HeLa). The GoIs can be expressed with or without certain tags for detection purposes and their expression is determined by standard techniques (e.g. western blot, pull-down assays, ELISA). Beside the expression of the GoI each target candidate can be knocked down by siRNA transfer if necessary. Respective standard procedures have been developed too.

Functional characterization

Most of the following functional evaluation assays are adapted to a 96-well format which allows a quick and easy detection of several different parameters. After the overexpression (or knock-down) of the GoIs the cells are harvested and screened for apoptotic mediators (e.g. activation of certain caspases, Bcl_2, Bcl_{XL}), cytotoxicity or proliferation as well as the secretion/activation of pro-inflammatory molecules (e.g. TNF-α, IL-1β, IL-6, IL-8, IL-12, IFN-γ, ICAMS) and pathways (e.g. NF-κB, STATs, MAP kinases, JNKs). A special focus is laying on the systematic pathway profiling which allows the activation detection of up to 20 different pro-inflammatory pathway proteins.

Standard Stimulation Plates

Beside the biological function of the GoIs another focal point is laying on the regulation of the GoIs under pro- and anti-inflammatory conditions. For this reason cells are incubated with certain cytokines such as TNF-α, IL-1β, IL-6, IL-10 etc. or bacterial antigenes (e.g. LPS, PGN, MDP) and the mRNA expression of the GoI is subsequently detected by Taqman assay. This approach often results into the formation of new gene and protein clusters which don't obviously belong to functionally similar gene families.

Murine infection models

In collaboration with the Forschungszentrum Borstel one goal of this project is the establishment of a murine infection system which can be used to predict potential side-effects of new drugs.

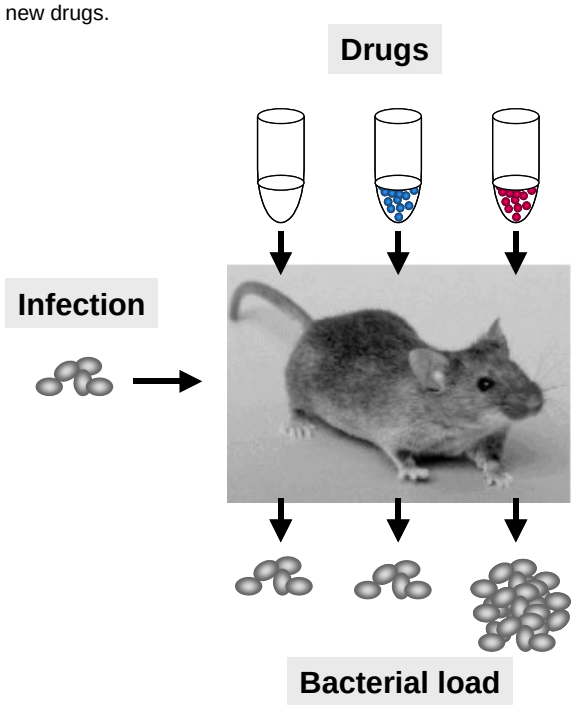

Fig 2: *Determination of side-effects (bacterial infections) after the application of drugs.*

For this purpose mice shall be infected with a defined amount of bacteria (*M. tubercolosis*) and will be treated with different doses of drugs. After certain timepoints the mice will be sacrificed and the bacterial load will be determined by

histological and molecular biological methods. The resulting bacterial titer will provide direct evidence for the putative side-effect potential of a certain drug at different concentrations.

Outlook

Systematic screening approaches are used to discover disease-associated genes and proteins which could function as new drugs or at least drugable targets. However, even if a new target is found, its value to serve as target for a therapy has to be determined before substantial drug screening approaches can be started.
Moreover, if new drugs have been designed it is difficult to make a reliable prediction about undesired adverse reactions during medications. Anti-inflammatory therapies often result into a down-regulation of the immune system which bears the potential for the development of severe secondary infections, e.g. tubercolosis. In order to save money and time during clinical development processes it is strongly eligible to find test systems which would allow an early evaluation of the side-effect potential of new drugs.

This project is designated to enhance drug discovery processes at two different levels. At the level of target evaluation it is intended to extend and improve already existing functional platforms by new techniques in order to increase the flow rates and to diminish the assay costs. At the level of drug evaluation a system shall be developed to determine drug side-effects. These assays will support early dicisions for the further pre-clinical and clinical development of certain new drugs.