

DIVISION OF CELL BIOLOGY II

ANTIGEN GENERATION AND PRESENTATION BY MHC CLASS MOLECULES AND CONTROL OF THE IMMUNE RESPONSE

Our aim is to understand the molecular mechanism of how the immune system detects infections and tumor cells. Understanding the control of the immune system also allows manipulation to improve immune responses for cancer control or to attenuate responses with autoimmunity. We also developed chemical biology tools to identify small molecules allowing manipulation and to integrate that with other screens. Ultimately, we aim at identifying target-lead combinations that can be further developed into strategies for manipulating immune responses and tumors. In this development program, the effects of compounds and siRNAs are considered in a broad context. By studying the cell biology of antigen presentation, we can understand the mechanism of many targets and some compounds in molecular detail. We use a large variety of state-of-the-art technologies such as high content-high resolution microscopy, lentiviral shRNA screens, chemistry and biophysical techniques to achieve our goal: translating insights in relevant biological systems into chemical tools for manipulation.

The turn-over of proteasomes The proteasome is a large abundant and very stable protein complex involved in the destruction of most intracellular proteins. How such stable protein complexes are destroyed is unclear but this provides interesting options for manipulation. We have introduced a color-switch cassette (developed with Dr F van Leeuwen, NKI) in one of the protein subunits of the proteasome, which allow us to follow the half-life (ie the decay of the green signal). Since the green signal also decays by dilution as the result of growth, we arrested yeast growth by food deprivation. Under these conditions, the proteasome left the nucleus and aggregated in the cytosol. Following food addition, the aggregates dissolved and the proteasome entered the nucleus again. We have introduced a yeast knock-out library in our yeast strain to identify the proteins involved in the various parameters. These yeast mutants are screened in a timed fashion and analyzed by confocal microscopy. We have established a semi-automatic microscopy system and automated image analyses and are currently completing the analyses of hits involved in the various systems. We aim to identify pathways and will transfer these to mammalian systems when the mammalian homologues are identified. This should yield new biology and potentially targets for further manipulation.

Cell Biology of anti-cancer compounds We have compared the cell biological activities of two topo-isomerase inhibitors; doxorubicin and etoposide. Using cell biological tools, we showed that histones were transiently leaving chromatin of non-mitotic cells only when cells were exposed to doxorubicin. This is due to the anthracyclin structure rather than double strand break formation as result of topo-II inhibition. Using FAIRE-Seq, we showed that histones are released from gene-



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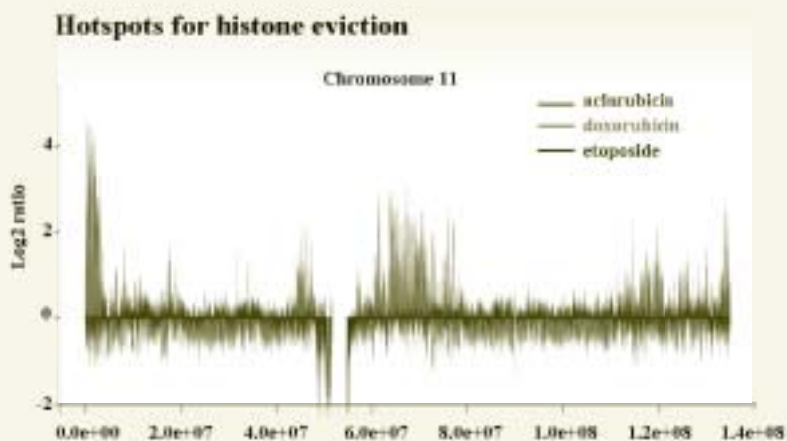


Figure 1: Genome-wide landscape of histone eviction induced by doxorubicin or aclarubicin treatment. Formaldehyde Assisted Isolation of Regulatory Elements followed by next generation sequencing (FAIRE-seq) is used to explore the whole human genome. Chromosome 11 is used as an example. Unique nucleosome free regions due to histone eviction are observed when cells were treated with doxorubicin or aclarubicin, compared to control cells. Etoposide treatment is similar to control situation because it could not evict histones.

Publications

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rich areas. As also H2Ax is released, doxorubicin – but not etoposide – strongly attenuates DNA repair of double strand breaks induced by the compounds. We show that the result of these manipulations is massive alterations of the transcriptome in tissue culture cells as well as in vivo. Especially the heart of mice is affected by doxorubicin alone, which may correlate to the altered transcriptome.

Using novel technology on an 'old' anti-cancer compound allows identification of novel cell biological mechanisms occurring in response to doxorubicin.

A systems analysis of MHC class II antigen presentation MHC class II molecules are expressed on so-called Antigen Presenting Cells, such as DC, monocytes and B cells and control almost all immune responses. Although various proteins have been defined as involved in MHC class II antigen presentation, a full description of these is lacking. We have performed a flow cytometry-based siRNA screen for factors controlling MHC class II expression and peptide loading. To place the 290 hits in pathways, we broke down the MHC class II pathway in two essential components: regulation by transcription and by cell biology. We determined higher order regulation of a transcriptional network controlling MHC class II expression by analysis of literature and of yeast two hybrid data that defined a new molecule in the TGFb pathway in control of MHC class II expression.

All hits were also tested in a microscopy-based assay where automated image analyses and computer-based machine learning were used to place cellular effects in clusters. These clusters probably represent pathways and this is considered the first step toward pathway definition. To test this, we studied the mechanism of MHC class II export in dendritic cells following activation signals. We selected the corresponding cluster and silenced the hits in immature DC while scoring for a matured phenotype and identified 8 hits that – when silenced – produced immature DC with a matured phenotype. We subsequently identified a novel pathway operational in human dendritic cells that couple a novel GTPase on MIIC compartments with myosin Ie motor protein control.

We now aim to further improve our data set by introducing the results of other data sets published in literature. In addition, a chemical library screen is performed and now integrated in the siRNA screen to define novel target-lead combinations. This integrated chemical biology approach should yield biological understanding and leads for manipulating MHC class II antigen presentation.

Bacterial infection, the PKB/Akt pathway and cancer We have studied the biology of intracellular infections using a combination of chemical biology and siRNA screening followed by a cell biological elucidation of pathways. We showed that inhibiting Akt1 was sufficient to activate phagosomal fusion with lysosomes and elimination of the bacteria, by manipulating Rab14. We have identified the effector for Rab14 that brings together a series of other Rab proteins to control intracellular transport. We have (in collaboration with Hermen Overkleeft, LIC, Leiden) generated a kinase compound library to improve our Akt1 inhibitors. This yielded a highly selective Flt-3 inhibitor that is active against a subset of ALL, and a reasonably selective Akt1 inhibitor with poor activity on Akt2. We are further improving these compounds and testing the Flt-3 inhibitors in xenotransplantation mouse models for ALL.

We repeated the infection experiments with a phosphatase siRNA library and place phosphatases in kinase networks. We identified the five subunits of PPP2 which controls Akt. We also identified different other phosphatases. As we performed a simultaneous chemical library screen for esterases and phosphatases, we aim at identifying new target-lead combinations with biological activities.

Activation of Akt is an important driver for cell transformation. We tested whether intracellular bacteria can induce transformation and infected MEFs with one or multiple pre-transforming mutations with *Salmonella* before culturing the cells in soft agar. Soft agar growth was detected provided Akt activation in MEFs with at least three pre-transforming mutations. This study is now expanded to other systems and molecular pathology to test the role of bacterial infections in cancer formation.