

## POSSIBLE MODEL FOR TESTING ERBB2 TARGETED THERAPIES

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**Keywords: ErbB2, transfected, therapy, characterization**

ErbB2 is a receptor tyrosine kinase underlying bad prognosis in several human malignancies. Trastuzumab, a humanized anti-ErbB2 antibody is a specific targeted therapy against these tumors, with a history of both success and a high rate of therapy resistance. The SADR cell line is a derivative of NIH3T3 fibroblasts stably transfected with the ErbB2 gene under the negative control of tetracycline.

We studied the age dependent (3<sup>rd</sup>, 10<sup>th</sup> and 20<sup>th</sup> passages) sensitivity of native and ErbB2-deprived (tetracycline treated) SADR cells to short (5 minutes) and long (3 days) treatments with 0.1 mg/ml trastuzumab in terms of proliferation, cell cycle, ErbB2 expression and phosphorylation, as well as apoptosis.

The proliferation rate upon ErbB2 deprivation by tetracycline decreased at all passages, coherent with the proliferation advantage from excess ErbB2. In these cells, trastuzumab did not decrease the proliferation further, nor did it affect the cell cycle, thus confirming specific trastuzumab action targeting ErbB2. In native SADR not treated with tetracycline, trastuzumab caused a G1-block that was correlated with the passage number, also enhanced short term ErbB2 phosphorylation, and decreased ErbB2 expression in the long run. While in the earliest passage minimal effects were observed upon trastuzumab treatment, by the 20<sup>th</sup> passage all ErbB2-related effects have manifested.

It is likely that the complete signaling and regulatory system around the ectopic ErbB2 evolves in time, resulting eventually in addition to the oncogene and sensitivity to its targeting. The experiments also draw attention to the stability problems of transfected cellular systems.

## N71 THE COMPLEMENT REGULATORY PROTEINS CD55 AND CD59 ARE ACTIVE COMPONENTS OF THE LYPOPOLISACCHARIDE RECOGNIZING COMPLEX OF DENDRITIC CELLS

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**Keywords: CD55, CD59, LPS, dendritic cell, TLR4**

Lipopolysaccharide (LPS) is the characteristic microbe-associated molecular pattern of Gram(-) bacteria that is recognized by CD14 as an LBP-LPS complex and signals through TLR4-MD2. Despite the low expression of membrane CD14 in conventional dendritic cells (DCs), LPS induces DC activation however, the cell type-specific pathway of LPS stimulation is still enigmatic.

**Objective:** The primary function of the CD55 and CD59 glycosylphosphatidylinositol (GPI)-linked membrane proteins is the inhibition of the complement system to protect antilicious cells from complement-mediated damage.

However, CD55 is also part of the LPS receptor complex in Monocytes (Heine et al. Eur J Immunol 2003) and CD59 is involved in LPS-mediated signal transduction of oral keratinocytes (Yamamoto et al. Cell Signal 2003). The goal of our work was to study the role of the complement proteins CD55 and CD59 in LPS signaling of monocyte-derived DCs.

**Methods:** The gene expression of CD55 and CD59 was measured by real time Q-PCR, protein expression was detected by flow cytometry. The expression and function of CD55 and CD59 proteins was inhibited by specific silencing RNA (siRNA) transfected to developing DCs by electroporation. The level of secreted cytokines in LPS-activated moDCs supernatants was measured by ELISA, the migration of mature moDCs was tested in Boyden chamber.

**Results:** Our results show that as compared to monocytes the expression of CD59 increases in the course of DC differentiation, whereas the expression of CD55 decreases parallel with that of the LPS-recognizing CD14 receptor. Inhibition of CD55 and CD59 expression or function significantly reduces the degree of LPS-induced activation of DCs monitored by the membrane expression of CD83, the secretion of inflammatory cytokines TNF- $\alpha$ , IL-6, IL-12, and the migratory capacity of mature moDCs.

**Conclusion:** These results show that the complement regulatory proteins CD55 and CD59 play an important role in LPS-mediated DC signaling, activation and effector functions. Moreover, the composition of the LPS-recognizing complex is reorganized during monocyte to DC transition. As both innocent and pathogenic microbes can trigger DCs through TLR4, the cell type-specific composition of the LPS receptor complex may transduce different stimulatory signals in various cell types.

## N72 HEAT SHOCK-INDUCED REARRANGEMENTS OF YEAST PROTEIN-PROTEIN INTERACTION NETWORK

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**Keywords: network, heat shock, yeast, module, adaptation**

In recent years biological data sets became exceedingly complex. Since most of these datasets have a high medical significance, their adequate interpretation is of high priority for potential therapeutic purposes. The analysis of the topology of the cellular networks constructed from these datasets may show novel changes at the systems biology level. We investigated the topological changes of yeast (*S. cerevisiae*) protein-protein interaction (PPI) network after heat shock. Overlapping modules of the yeast interactome were determined using NodeLand method developed recently in our research group ([www.linkgroup.hu/modules.php](http://www.linkgroup.hu/modules.php)). Changes in link weights of the exponentially growing yeast cells after heat shock were modeled using mRNA microarray data and converting the changes in mRNA levels to changes in the probability of protein-protein interactions.

Our results showed a partial disintegration of PPI network: protein modules having distinct functional annotations became