Transglutaminase 2 as an essential regulatory factor of neutrophil granulocyte differentiation

Potential contribution in retinoic acid syndrome

by Krisztián Csomós

Department of Biochemisrty and Molecular Biology
University of Debrecen

Doctoral school of
Molecular Cell and Immune Biology

Supervisor: Dr. Zoltán Balajthy
Department of Biochemisrty and Molecular Biology
University of Debrecen

DEBRECEN, 2010

Therapy of acute promyelocytic leukemia (APL) primarily consists of an *all-trans*-retinoic acid/ATRA-based treatment, which results in terminal differentiation of leukemic cells toward neutrophil granulocytes. However this differentiation-induced therapy is often accompanied by organ infiltration of differentiating leukemic cell leading severe hyper-inflammatory response in lung as an even lethal side effect of ATRA-treatment, called retinoic acid syndrome (RAS). Administration of ATRA leads to massive changes in gene expression in APL cells, including down-regulation of cell proliferation related genes and induction of genes involved in immune functions of neutrophil granulocytes. One of the most induced genes by ATRA in APL NB4 cells is transglutaminase 2 (TG2).

We have demonstrated that transglutaminase 2, after its induction, partially translocates into the nucleus, associates to the chromatin and is able to modify nuclear proteins by its acil-transferase activity during the differentiation process. The transglutaminase-catalyzed cross-link content of both the cytosolic and the nuclear protein fractions increased while NB4 cells underwent cellular maturation. Inhibition of cross-linking activity of TG2 by monodansylcadaverin in these cells led to diminished nitroblue tetrazolium (NBT) positivity, production of less superoxide anion, and decreased expression of $gp91^{phox}$, the membrane-associated subunit of NADPH oxidase. Neutrophils isolated from TG2-/- mice showed diminished NBT reduction capacity, reduced superoxide anion formation, and down-regulation of the $gp91^{phox}$ subunit of NADPH oxidase, compared with wild-type cells. These results clearly suggest that TG2 may modulate the expression of genes related to neutrophil functions and is involved in the process of differentiation of neutrophil.

To further investigate the role of TG2 in the differentiation process, RNA interference-mediated stable silencing of TG2 in NB4 cells (TG2-KD NB4) coupled with whole genome microarray analysis was performed. Our experiment revealed that TG2 contributes to the expression of a numerous ATRA-regulated genes. In the TG2-KD NB4 cells during ATRA-induced differentiation large number of genes related to neutrophil granulocyte function stayed partially suppressed. Down-regulation of these genes led to reduced adhesive, migratory and phagocytic capacity of neutrophils and less superoxide production. ATRA-controlled down-regulation of those genes, which are involved in cell cycle control and cell proliferation held at higher expression level and found to be manifested in a higher proliferative rate of TG2-KD NB4 cells. Since we observed that the induction of CC-chemokines (CCL2, -3, -22, -24), which are responsible for the development of retinoic acid syndrome (RAS) in ATRA treated APL patients, were significantly lesser in TG2-silenced NB4 cells, significance of TG2 may have far-reaching consequences in clinical aspect of ATRA treatment APL patients.

Based on our results we suppose a complex regulatory effect of TG2 upon the retinoic acid-mediated differentiation of myeloid cells and propose that a reduced expression of TG2 in differentiating APL cells may suppress effector functions of neutrophil granulocytes and therefore moderate ATRA-induced hyper-inflammatory response in RAS.

Transglutaminase 2, gene expression, acute promyelocytic leukemia, cell differentiation, neutrophil granulocyte

transzglutamináz 2, génexpresszió, akut promielocitás leukemia, sejt differenciáció, neutrophil granulocita