Karolinska Institutet

Institutionen för Onkologi-Patologi

ROR1-a Novel Receptor Tyrosine Kinase with Unique Therapeutic Potentials in Chronic Lymphocytic Leukemia

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen
vid Karolinska Institutet offentligen försvaras i
Cancer Centrum Karolinskas Föreläsningssal R8:00,
Karolinska Universitetssjukhuset Solna

Onsdagen den 2 november 2011, kl 10.00

av
Amir Hossein Daneshmanesh

Huvudhandledare:
Docent Hodjattallah Rabbani
Karolinska Institutet
Institutionen för Onkologi-Patologi

Bihandledare:
Professor Håkan Mellstedt
Karolinska Institutet
Institutionen för Onkologi-Patologi

Professor Anders Österborg
Karolinska Institutet
Institutionen för Onkologi-Patologi

Fakultetsopponent:
Professor Anders Rosén
Linköpings Universitet
Institutionen för Cellbiologi

Betygsnämnd:
Docent Ola Söderberg
Uppsala Universitet
Institutionen för Genetik och Patologi

Professor Stig Linder
Karolinska Institutet
Institutionen för Onkologi-Patologi

Professor Robert Hast
Karolinska Institutet
Institutionen för Hematologi

Stockholm 2011

ROR1-a Novel Receptor Tyrosine Kinase with Unique Therapeutic Potentials in Chronic Lymphocytic Leukemia

Abstract

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of small B lymphocytes in blood, bone marrow, lymph nodes and other lymphoid tissues. CLL is the most common leukemia in the Western world. Despite significant advances in understanding the pathogenesis, CLL is still a disease with no available cure.

Receptor tyrosine kinases (RTKs) are a large family of cell-surface receptors participating in crucial cellular processes including proliferation, differentiation, cell-cell interaction, metabolism, signaling, migration and cell survival. More than half of the RTK families are overexpressed or mutated in different forms of human cancer. RTKs are multifunctional therapeutic targets and novel RTKs in cancer have been pursued extensively as a goal for targeted therapies. ROR1 belongs to one of twenty families of RTKs. It is a survival kinase and acts as a receptor for Wnt5a protein.

Gene expression profiling studies have shown that ROR1 was upregulated in CLL patients. Characterization of ROR1 expression in CLL and the study of its functional role for possible therapeutic targeting was the driving force of this thesis.

In the first study we investigated the expression pattern of ROR1 in CLL. All CLL patients (n=18) expressed ROR1 both at gene and protein levels but none of the healthy donors. CLL patients showed a ROR1 surface expression in the range of 36–92%. Western blot analysis revealed two ROR1 bands of 105 and 130 kDa. Mutation analysis of the ROR1 gene showed no major genomic aberrations. FISH analysis of PBMC from 3 CLL patients showed no rearrangement in the 1p region.

The second study was conducted to examine the effects of siRNAs specifically silencing ROR1 and fibromodulin (FMOD) in CLL cells. siRNA treatment induced a specific reduction (75–95%) in FMOD and ROR1 mRNA expression. Western blot analysis for ROR1 and FMOD demonstrated that the proteins were significantly downregulated 48 h after siRNA treatment. Silencing of FMOD and ROR1 resulted in a statistically significant apoptosis of CLL cells but not of B cells from normal donors.

In the third study, five ROR1 monoclonal antibodies were raised against extracellular domains of ROR1 to investigate the in vitro apoptotic effects on CLL cells. All five mAbs induced apoptosis of CLL but not of normal B cells in the absence of complement or immune effector cells. Most effective were mAbs against CRD and KNG, being superior to rituximab in vitro. Cross-linking of the anti-ROR1 mAbs using F(ab´)2 fragments of anti-Fc antibodies significantly augmented apoptosis. Two of the mAbs induced complement-dependent cytotoxicity similar to that of rituximab.

The fourth study was aimed at investigating ROR1 and ROR2 expression in hematological malignancies of lymphoid and myeloid origins. The results showed a statistically significant variation in the expression of ROR1 in various hematological malignancies. No expression of ROR2 was detected in hematological malignancies tested and PBMC of healthy donors. A statistically significant higher expression of ROR1 was detected in progressive compared to non-progressive CLL patients. ROR1 expression was shown to be stable overtime.

In conclusion ROR1 was found to be ectopically expressed in CLL. Given the successful history of RTKs targeted therapies in cancer, ROR1 might be a novel potential therapeutic target structure in CLL.

ISBN 978-91-7457-504-0