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**Towards New Therapeutic Targets:  
Identification of Novel Tumor Markers  
in Chronic Lymphocytic Leukemia**

AKADEMISK AVHANDLING

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# **Towards New Therapeutic Targets: Identification of Novel Tumor Markers in Chronic Lymphocytic Leukemia**

## **Abstract**

Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in the Western world and is caused by an abnormal accumulation of B lymphocytes in peripheral blood, bone marrow and lymphoid organs. It is a disease mainly of adults.

The clinical outcome of CLL may differ significantly. Some patients have an indolent leukemia with long survival, while others experience an aggressive disease, with an acute need for treatment. At present, no treatment regimen can be considered curative. Novel therapeutic approaches are required. Those aimed at directing the body's natural defense system against the tumor cells require well characterized targets that are exclusively expressed by the tumor cells.

In 2001, microarray studies revealed **FMOD**, a member of the Small Leucine Rich Proteoglycan family (SLRP) and **ROR1**, a member of the Receptor Tyrosine Kinase (RTK) family, as two genes being overexpressed in CLL compared to healthy controls. SLRPs are normally expressed and secreted into the extracellular matrix of collagen-rich tissues. They interact with collagen and participate in signaling regulation by the interaction with integrins, growth factors and their receptors. RTKs are transmembrane receptors for growth factors, cytokines and hormones and play important roles in cellular processes including proliferation, differentiation, migration, metabolism and survival.

The ectopic expression of FMOD and ROR1 in CLL was unexpected and was the prelude to this thesis. FMOD is located on chromosome 1 adjacent to two other members of the SLRP family; **PRELP** and **OPTC**.

In this project, we investigated FMOD, PRELP, OPTC and ROR1 at the gene as well as protein levels and their expression in CLL compared to healthy individuals and other hematological malignancies. We also investigated the effect of siRNA silencing of FMOD and ROR1 in CLL and normal control cells.

FMOD, PRELP, OPTC and ROR1 were expressed in all CLL patients but not in normal controls. ROR1 was detected on the surface of CLL cells, which corresponds to the natural ROR1 localization. PRELP and OPTC, on the other hand, seemed to be abnormally retained within the CLL cells, rather than secreted. The PRELP and OPTC proteins expressed in CLL were further investigated and found to be differentially glycosylated compared to their normal counterparts. The molecular weight of the detected PRELP and OPTC corresponded to completely unglycosylated core proteins. PRELP was detected in the cytosol of CLL cells, while CLL OPTC was found in the nucleus and endoplasmic reticulum.

Using siRNA technology, FMOD and ROR1 were efficiently downregulated which resulted in apoptosis of CLL cells but not of B cells from healthy donors. This suggests that FMOD and ROR1 may be important for the survival of CLL cells.

In conclusion, four genes, FMOD, PRELP, OPTC and ROR1, were found to be ectopically expressed by CLL cells. At least two of these genes, FMOD and ROR1, may be important for CLL cell survival. The reason for the aberrant expressions is not yet known, but once elucidated it may contribute to the understanding of the pathogenesis of CLL. Also, these novel markers might be suitable targets for future immunotherapy in CLL.