New Doctorial Cancer Research

Identification and Characterization of Novel Genes Expressed in Neoplastic Human B Cells

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Non-Hodgkin’s lymphoma (NHL) is a frequent malignant disease with increasing incidence rate (~ 600 new cases per year in Norway). Accounting for ~ 20% of the cases, follicular lymphoma (FL) represents the most common low-grade NHL. FLs are of B cell origin, generally incurable, and frequently transform histologically to high-grade diffuse large B cell lymphoma (DLBCL). Transformation is generally correlated with poor prognosis. The involved molecular mechanisms are currently largely unknown. The present study aimed at the identification of novel, differentially expressed genes in patient matched biopsies of FL and DLBCL. The characterization of genes expressed in DLBCL but not the preceding FL is thought to increase the understanding of disease progression. The Doctorial work includes four articles.

Initial validation of a subtraction methodology [cDNA representational difference analysis (cDNA RDA)] on human leukemia and lymphoma B cell lines identified expected gene expression differences and, in addition, led to the characterization of the human endogenous retrovirus gene HERV-H/F expressed in leukemia-derived cell lines of B lymphoid and myeloid origin. Independently from our study, a childhood acute lymphoblastic leukemia related expression of HERV-H/F expression has been reported suggesting its potential application as a tumor/residual disease marker.

The application of cDNA RDA on lymphoma biopsies identified two novel genes: the Immunoglobulin-like domain containing receptor 1 gene (ILDR1) and the centrosome/microtubule associated protein 1 gene (CSPP1). ILDR1 encodes for a multimeric receptor that can be assembled by different isoforms. Interestingly, a lymphoma associated expression of one of its isoforms was noted upon examination of a limited set of biopsies. Two isoforms encoded by CSPP1 have been characterized, differing in affecting cell cycle progression through G1 and mitosis. Independently, CSPP1 mRNA overexpression has been correlated to poor prognosis in de novo DLBCLs; that is, DLBCLs from patients that lack a history of FL. We show that overexpression of CSPP isoforms disturbs normal spindle assembly and may thus promote numerical chromosome instability. The suggested involvement in the G1 to S phase progression appears of particular importance because it adds to the emerging role of the centrosome in cell cycle control.

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The molecular mechanisms governing histological transformation of low-grade FL to high-grade DLBCL are largely unknown. Initial validation of a cDNA subtraction methodology on cell lines and its subsequent application on patient matched biopsies of FL and DLBCL identified three novel genes with B cell malignancy associated expression: HERV-H/F (B cell leukaemia), ILDR1, and CSPP1 (DLBCL). Expression of CSPP1 has been shown, independently of our findings, to be associated with poor prognosis in DLBCL. Kaplan-Meier estimates of overall survival of patients with de novo DLBCL with below- (green) and above-median (purple) mRNA expression levels of CSPP1 (Unigene cluster Hs.370147, data from Ref. 6. Twenty-seven out of 240 patients were excluded due to missing values. Inclusion of these 27 patients in the below-median expression group further increased the significance to p = 0.025 (data not shown). Ectopic expression of EGFP tagged CSPP in the human embryonic kidney cell line Hek293T impairs cell cycle progression in G1 phase and in mitosis as shown by DNA histograms of CSPP-transfectants five days posttransfection (upper panel). CSPP-EGFP expressing cells show increased tetraploid (4n) and > 4n populations indicative for aneu/polyplody compared to controls (upper panel, left). Furthermore, CSPP-EGFP expression perturbs cell cycle progression though G1 phase as revealed by the retention of a diploid (2n) population upon treatment with the mitotic drug Colcemide 24 hr prior to analysis (upper panel, right). CSPP-egfp expressing mitotic cells are characterized through the expression level dependent aberration of bipolar spindle formation and chromosome congression (lower panel). At very low expression levels normal metaphases are observed as shown by immunofluorescence staining 24 hr posttransfection (CSPP-EGFP, green; centrosomes (anti-γ-tubulin), red; DNA, blue). Increased expression leads to formation of predominantly multipolar spindles and lack of chromosome congression.

REFERENCES


**Comment by Stephen Doxsey**

The thesis consists of an introduction giving an overview of B cell malignancies, in particular B-cell lymphomas, as well as general discussion of the main methods applied and published in the four included articles. The work is part of a larger effort of the laboratory where it has taken place to molecularly characterize different stages of B cell lymphomas as well as to develop tools enabling this characterization. The work is centered around the characterization of certain genes originally identified by the technique of cDNA representational difference analysis, which has been used in attempts to identify novel genes expressed in particular cell types. The introduction and general discussion, including a more specific discussion of the methods applied, is generally well written and serves its purpose of putting the published work into perspective.

In total, three novel genes with a potential role in B cell malignancies have been identified: HERV-H/F in leukemia; ILDR1 and CSPP1 in lymphoma. The articles represent the initial characterization of these genes and its gene products. Particular efforts have been made in the functional characterization of two isoforms encoded by the centrosome/spindle pole associated protein 1 gene (CSPP1 gene) suggesting their involvement in the control of cell cycle progression at the G1/S phase transition and microtubule organization in mitosis. The correlation between CSPP1 mRNA overexpression and poor prognosis in a larger set of de novo DLBCLs in conjunction with the mitotic defects observed upon overexpression in vitro, suggests CSPP to be added to the list of centrosomal proteins involved in cell cycle control and cancer development/progression.