Karolinska Institutet

Institutionen för Onkologi-Patologi

Regulation of Molecular Processes in Diffuse Large B-cell Lymphoma

AKADEMISK AVHANDLING

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

Fredagen den 24 maj 2013, kl 09.30

av

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Stockholm 2013

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Abstract

The molecular understanding of diseases has advanced rapidly due to the use of gene expression profiling. However, these methods have been hampered by the limitation to use frozen tissue specimens. Formalin fixation and paraffin embedding (FFPE) is a standard procedure for long time storage of tissues. FFPE tissues are available in large numbers and are of value for molecular research with the main challenge of low RNA quality compared to fresh frozen (FF) tissues. This thesis showed important aspects on laboratory methods of gene expression using FFPE material analyzing gene regulation and environmental factors in patients with Diffuse Large B-cell Lymphoma (DLBCL). In **Study I**, we evaluated RNA extraction and gene expression of long-term preserved FFPE Non-small Cell Lung Cancer (NSCLC) specimens using quantitative PCR (qPCR) and microarray. High quality gene expression signatures could be recognized in long time stored FFPE tissues. According to the results of Study I, FFPE tissues were further used in Studies II, III and IV.

Different countries of the world have varying prevalence of microbial infections. It should be of interest to study patient populations originating from regions with different infectious and environmental exposures with the same disease. Sweden and Egypt are countries defined as low and high endemic infectious disease areas respectively. DLBCL is the most common type of Non Hodgkin's Lymphoma (NHL) and accounts for approximately 40% of newly diagnosed lymphomas worldwide. The ABC subgroup of DLBCL (ABC DLBCL) has a poor prognosis with short survival. NHL has been associated to viral infections as Epstein Barr virus (EBV) and Hepatitis viruses B and C (HBV, HCV). To understand if differences in environmental exposure are associated to the activated B-cell type (ABC) of DLBCL, we analyzed the expression of genes, regulatory factors and microbial agents of Swedish and Egyptian ABC DLBCL patients using microarrays. In **Study II**, we compared the global gene expression profiles of Swedish and Egyptian patients. Signal transducer and activators of transcription 3 and 5 (STAT3 and STAT5b) were differently expressed. STAT3 was significantly upregulated in Swedish compared to Egyptian patients and controls. The opposite expression patterns was demonstrated for STAT5b. The difference in STAT3 and STAT5b expression was confirmed at the protein level. Based on these results, we investigated microRNA (miRNA) expression profiles in **Study III**. miRNAs are non coding RNAs targeting mRNA modulating their expression at the post-transcriptional level. We found that miRNA-1234 (miR-1234) was significantly upregulated in Egyptian compared to Swedish patients. The expression level of miR-1234 correlated inversely to the expression of STAT3. Furthermore, the Stat3 protein was downregulated in cells transfected with miR-1234, suggesting that STAT3 might be a potential target for miR-1234. In **Study IV**, we analyzed the presence of microbial agents in Swedish and Egyptian ABC DLBCL patients using a microbial detection array (MDA). JC polyoma virus (JCV) was detected in both Swedish and Egyptian patients and the complete HBV genome in Egyptian patients. Study IV supports the notion that viral agents such as JCV and HBV may be involved in the tumorigenesis of DLBCL in high infectious disease regions. ABC DLBCL patients originating from areas with different environmental exposures have altered gene and miRNA expression profiles and a different viral load, which may be of importance for the development of ABC DLBCL. STAT3 may be regulated by miRNA and associated to the presence of viral infections. These results may be of potential importance for the development of STAT targeted therapy.

ISBN 978-91-7549-107-3