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Inna Gordiienko **Lecture 5**

SAP DEFICIENCY IN X-LINKED LYMPHOPROLIFERATIVE DISEASE TYPE 1 AFFECTS B-CELL DIFFERENTIATION

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X-linked lymphoproliferative disease type 1 (XLP1) belongs to genetically determined primary immunodeficiency syndromes with mutations in SH2D1A/DSHP/SAP gene. The dramatic increase of the risk of B-cell lymphoma development in XLP1 patients is linked not only to SAP deficiency of NK, NKT, and T cells, but probably to the impairment of B cell differentiation. Aim: To analyze the cell surface phenotype, functional characteristics, receptor-mediated Akt/PKB and ERK1/2 activation, and expression of several transcription factors in EBV-transformed B-lymphoblastoid cell lines from XLP1 patients (XLP B-LCLs) in comparison with conventional B-lymphoblastoid cell lines (B-LCLs). Studies were performed on EBV-transformed XLP B-LCLs IARC 739, XLP-D, XLP-8005, SC-XLP and RP-XLP; B-LCLs T5-1, 6.16, RPMI 1788, and MP-1. MTT assay, Annexin V binding and trypan blue exclusion tests, Q-PCR, western blot and flow cytometry analysis were used.

EBV-transformed XLP and conventional B-LCLs express B-lineage specific cell surface markers. Expression levels of CD19, CD40, CD48, CD80, CD95, CD150 and IgM were similar in both XLP and conventional B-LCLs. All tested cell lines were heterogeneous by IgD expression and did not express CD27. However, CD20, CD38 and CD86 expression levels were upregulated on XLP B-LCLs. Signals via CD150 and CD40 receptors had different effect on modulation of CD95-mediated apoptosis in XLP and conventional B-LCLs. In conventional B-LCLs, CD40 ligation before of together with CD95 partially rescued cells from apoptosis. However, signals via CD40 were synergistic with CD95-mediated apoptosis in XLP B-LCLs IARC-739 and XLP-D. CD150 and CD95 co-ligation augmented the number of apoptotic cells (compared to the effect of CD95 alone) in all tested B-LCLs, but did not alter the level of CD95-induced apoptosis in XLP B-LCLs. Moreover, while combination of signals via CD40 and CD150 did not affect the level of CD95-mediated apoptosis in B-LCLs, in XLP B-LCLs CD150 ligation did not reduce synergistic effect of CD40 and CD95. The major distinct feature of XLP B-LCLs was unresponsiveness to proliferative signals triggered by CD40 or colligation of BCR with CD150. SAP deficiency in XLP B-LCL did not abrogate CD150-mediated Akt and ERK1/2 phosphorylation. CD150 crosslinking on XLP B-LCL IARC 739 led to two waves of ERK1/2 phosphorylation. CD150 signaling in XLP B-LCL diminished the amplitude of IgM-mediated Akt phosphorylation. SAP+ and SAP- conventional B-LCLs have differences in kinetics and amplitude of receptor-initiated Akt phosphorylation. Analysis of transcription factors profile revealed a significantly reduced IRF4, IRF8 and PU.1 expression levels in XLP B-LCLs with SAP deficiency. This is a distinguishing feature of XLP B-LCLs that may affect B cell differentiation in XLP1 patients.

Our study provides new evidence for intrinsic defects in B-lymphoblastoid cell lines from patients with X-linked lymphoproliferative disease type I that affects B-cells activation, apoptosis, proliferation and differentiation.

Svetlana Sidorenko **Lecture 1**

EXPRESSION OF CD150 IN TUMORS OF THE CENTRAL NERVOUS SYSTEM: IDENTIFICATION OF A NOVEL ISOFORM

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CD150 (IPO3/SLAM) belongs to the SLAM family of receptors and serves as a major entry receptor for measles virus. CD150 receptor is expressed on normal and malignant cells of immune system. However, little is known about its expression outside the hematopoietic system, especially in tumors of the central nervous system (CNS). In human glioma cell lines we identified a novel CD150 splice isoform (nCD150) containing 83 bp insert, derived from a previously unrecognized Cyt-new exon located 510 bp downstream of the transmembrane region. This insert, which is a specific feature of primate *CD150* gene, results in the reading frame shift and formation of a cytoplasmic tail lacking any known signaling motifs. The predicted cytoplasmic tail of nCD150 counts 94aa, while mCD150 cytoplasmic tail is 72aa in length. Both mCD150 and nCD150 cDNAs did not contain any mutations, and leader sequence was unmodified. Our immunohistochemical studies revealed CD150 expression in 77.6% of human primary CNS tumors, including glioblastoma, anaplastic astrocytoma, diffuse astrocytoma, ependymomas, and others, but not in human normal brain tissues. CD150 was expressed in the cytoplasm, but not on the surface of human glioma cells, and colocalized with endoplasmic reticulum and Golgi complex. nCD150 mRNA was expressed in all tested samples and at high level by primary glioblastoma cells NCH92 and diffuse astrocytoma tumor sample. However, expression of CD150 splice isoforms' mRNAs encoding conventional cytoplasmic tail was very low or practically undetectable. The nCD150 isoform was also expressed in normal and malignant B lymphocytes, primary T cells, dendritic cells and macrophages, however in glioma cells nCD150 was a predominant CD150 isoform. Overexpression either nCD150 or mCD150 isoforms upon transfection of U87 glioma and HEK293T cell lines resulted in cell surface expression of both CD150 isoforms. Moreover, similarly to mCD150, cell surface nCD150 could serve as measles virus entry receptor. Thus, we identified a novel nCD150 isoform that is highly expressed in tumor cells of glial origin. CD150 expression in CNS tumors can be considered a new diagnostic marker and potential target for novel therapeutic approaches.

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