Abstract #51 | Poster

Functional role of AKT isoforms in Hodgkin Lymphoma

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The PI3K/AKT pathway is one of the most important factors in intracellular signal transduction, making it an essential component in the genesis of many cancer types. Even though previous studies examined the role of constitutive activation of this pathway in Hodgkin lymphoma, the functional role of the three distinct isoforms of the serine/threonine kinase AKT in the formation of the lymphatic cancer disease was not studied before. In this regard, the four established Hodgkin lymphoma cell lines L428, KMH2, HDLM2, and U-HO1 were examined within this study.

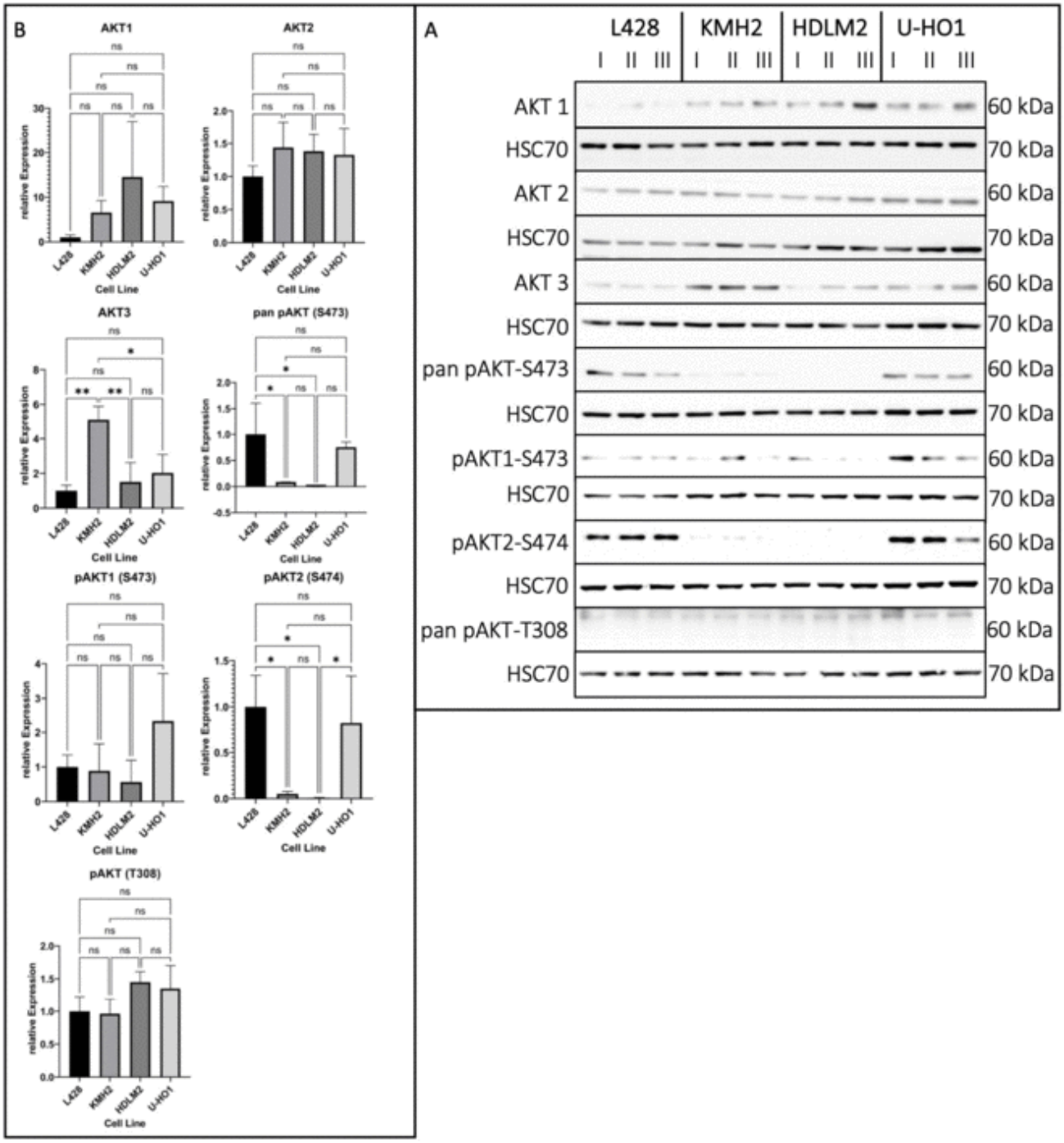
In all four cell lines, the expression of all three AKT isoforms, namely AKT1, AKT2 and AKT3, was proven by Western blot and an increased expression of AKT3 was detected in the KMH2 cell line. Measurement of the kinase activity revealed a correspondingly increased relative activity of AKT3. However, the highest specific AKT3 activity was determined in the U-HO1 cell line.

Using AKT isoform-specific knockdowns, a biological function of all three AKT isoforms for the proliferation of the Hodgkin lymphoma cell lines L428 and HDLM2 was shown, whereas knockdowns of AKT2 and AKT3, but not of AKT1, led to a decreased proliferation in U-HO1 cells. Surprisingly, the downregulation of AKT2 in the KMH2 cell line led to increased proliferation of the cells.

Furthermore, constitutive phosphorylation of AKT at T308 and S473 was detected in all examined Hodgkin lymphoma cell lines as well as the phosphorylation of the indirect or direct AKT downstream targets BAD at S136, S6 at S240/S244 and mTOR at S2448.

A decreased PTEN expression as well as a constitutive phosphorylation of STAT5 at T694 and a concluding activation of the JAK-STAT pathway were identified as possible reasons for the constitutive activation of the PI3K/AKT pathway in the HDLM2 cell line. The observed correlation between the phosphorylation of AKT at S473 and the phosphorylation of ERK 1/2 at T202 and Y204 points to a similar regulation of the PI3K/AKT pathway and the RAS/MAPK pathway in the examined Hodgkin lymphoma cell lines.

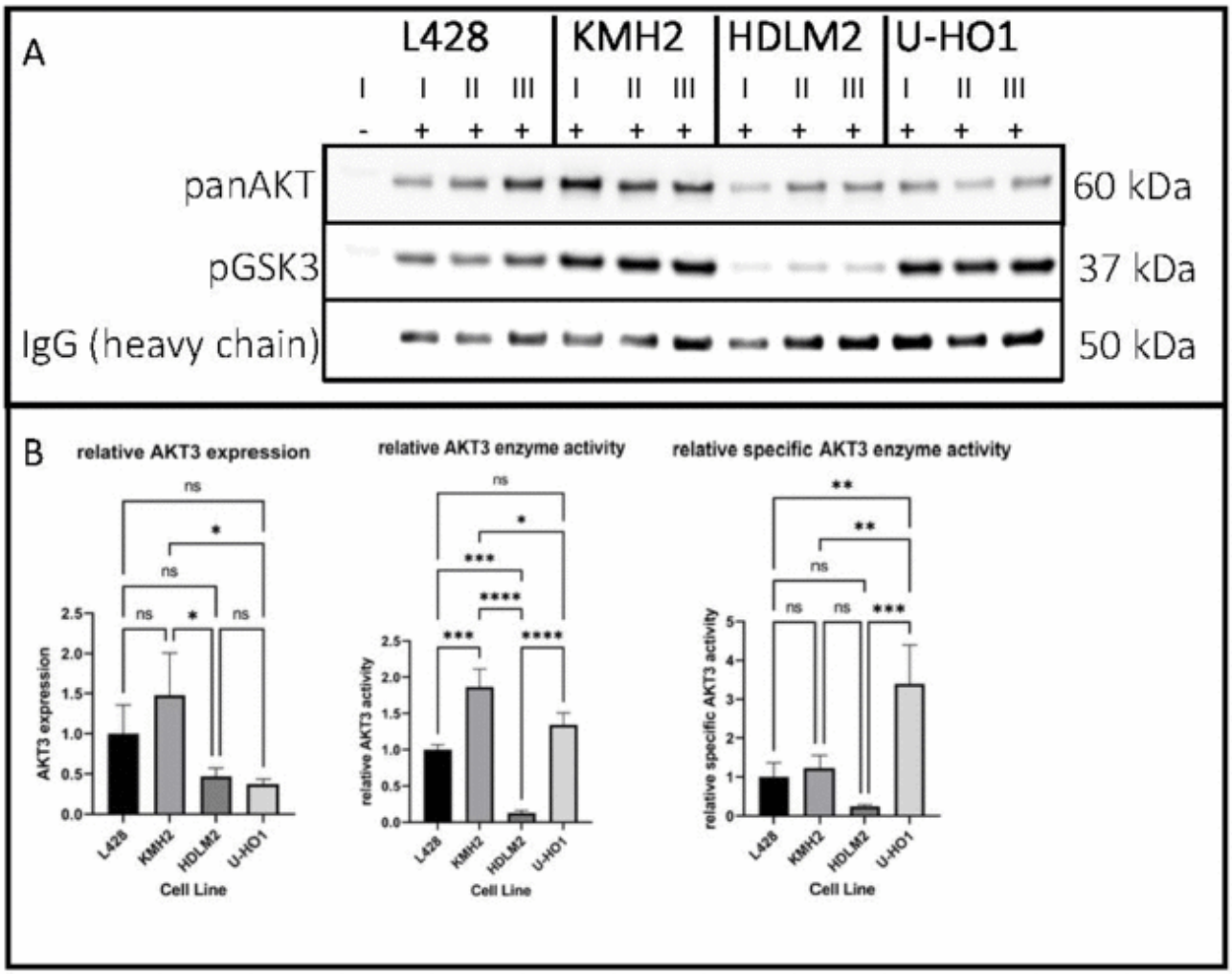
Altogether, the results of this study show a differential activation and biological function of the three AKT isoforms in Hodgkin lymphoma cell lines. These results should now be validated by further experiments on primary Hodgkin lymphoma as well as mouse models *in vivo*.



Expression of AKT isoforms in HL cell lines

A. Expression was analysed by Western Blot. 40 µg of protein were loaded in each lane and HSC70 was used as a loading control. Cell lines were analysed in biological triplicates. *B.* The bands were

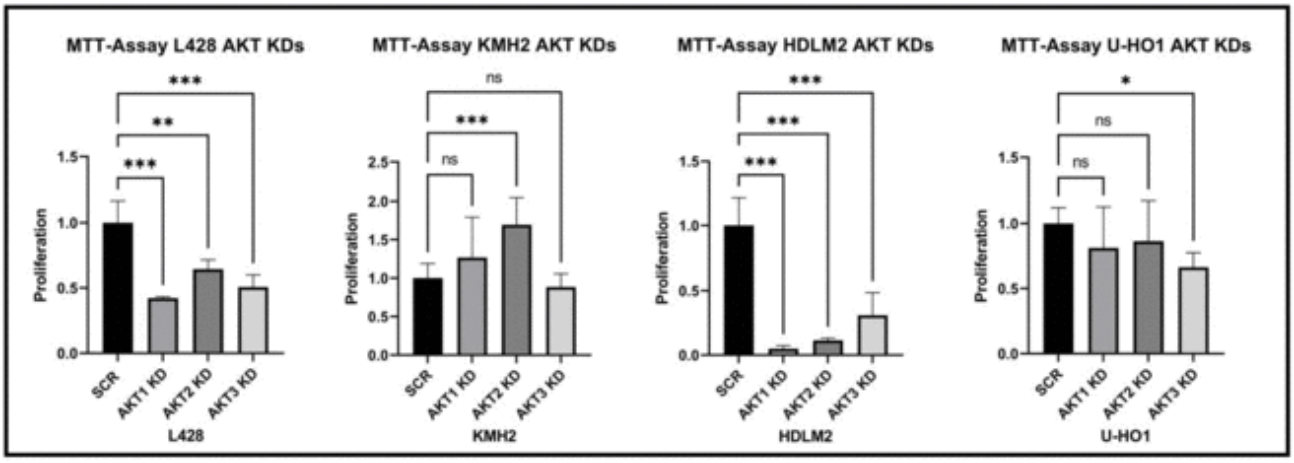
normalized using the signal of the loading control. One-way ANOVA was used for analysis of significant differences, n=3, ns: not significant, *: p0.05, **: p0.01, ***: p0.001, ****: p0.0001.



Kinase activity of AKT3 in HL cell lines.

A. Results of the kinase assay was analysed by Western Blot.

B. The relative AKT3 expression was calculated using the panAKT signal, the relative AKT3 activity was calculated using the pGSK3 band and the specific enzyme activity was calculated as the quotient of both values. One-way ANOVA was used for analysis of significant differences, n=3, ns: not significant, *: p0.05, **: p0.01, ***: p0.001, ****: p0.0001.



Proliferation of HL cell lines with AKT knockdowns

Proliferation was analysed by MTT assay. For the KMH2 and the U-HO1 cell line the data is combined from two experiment. The distinct results of the first and the second experiment can be seen in the attachments. One-way ANOVA was used for analysis of significant differences, L428/HDLM2: n=3, KMH2/U-HO1: n=9, ns: not significant, *: p0.05, **: p0.01, ***: p0.001, ****: p0.0001.