EXPRESSION OF PHOSPHORYLATED AKT AND PI3K KINASES ISOFORMS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

M. Lourou, E. Papakonstanti, M. Pesmatzoglou, E. Stiakaki

Department of Pediatric Hematology/Oncology, University Hospital of Heraklion & Biochemistry Laboratory Medical School

University of Crete, Heraklion Crete, Greece

Introduction

Acute lymphoblastic leukemia (ALL) is the most common neoplastic disease in children and represents 30% of all childhood cancers. Although 80% of ALL patients are long term survivors, there is still a percentage of resistant or relapsed disease. The identification of genes and molecular pathways that are involved is crucial for targeted therapy design.

The intracellular signaling pathway PI3K/AKT gets induced by growth factors and plays an important role in triggering many biological processes of the cell such as cell growth, metabolism, proliferation, survival, metabolism of insulin, protein synthesis and apoptosis. Upon growth factor stimulation, PI3K is recruited to the plasma membrane and activated. PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate to generate phosphatidylinositol-3,4,5-triphosphate. Akt binds to phosphatidylinositol-3,4,5-triphosphate on the plasma membrane where Akt is activated by phosphorylation at threonine308 (T308) and serine473 (S473) residues.

Recently it has been found that changes in the expression and mutations in many molecules of that pathway are involved in tumor genesis, which makes them an attractive target for cancer therapy.

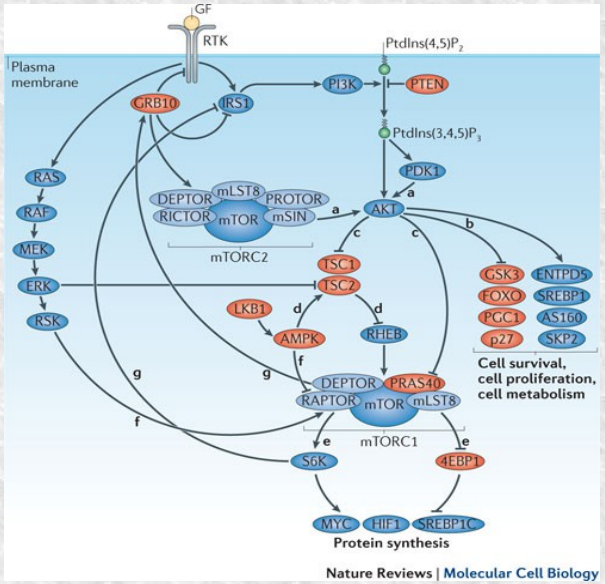

Figure 1. PI3K/AKT pathway

Aim

To investigate the ex pression of the proteins P110β, P110δ and P-Akt in leukemic cells of bone marrow from children with ALL at diagnosis and lymphomononuclear cells in remission, compared to the expression in lymphomononuclear cells from bone marrow of children with solid tumors to identify genes involved and correlated with the prognosis and the design of targeted therapy .

Patients and Methods

Protein extraction from:

- lymphoblasts of 32 children diagnosed with ALL
- lymphomononuclear cells of 21 children in remission
- lymphomononuclear cells from 20 children with solid tumors without bone marrow involvement.

200μgr of protein:

- were analyzed with acrylamide gel (Western blot)
- transfered to PVDF membrane
- detection of P-Akt, P110β and P110δ with Amersham ECL Prime.

The analysis of the results was done with Image Lab (Figure 2).

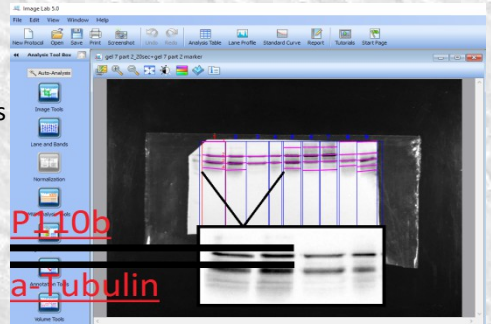

Figure 2. P110δ and a-Tubulin on PVDF membrane after incubation with Amersham ECL Prime.

Results

	P-AKT			P110β			P110δ		
	Diagnosis (n=32)	Remission (n=21)	Controls (n=20)	Diagnosis (n=32)	Remission (n=21)	Controls (n=20)	Diagnosis (n=32)	Remission (n=21)	Controls (n=20)
% of expression	84.4%	100%	90%	50%	61.9%	55%	87.5%	90.5%	70%
Median±standard deviation	0.210±6.849	0.857±2.138	0.64±1.049	0.291±1.067	0.122±0.386	0.13±1.134	0.330±3.779	1.057±2.048	0.748±0.619
p	0.115			0.947			0.03		

Table 1. Statistic analysis results

Statistically significant difference was found at the expression of P110δ between children diagnosed with ALL and children in remission (1.63±0.7 vs 1.73±0.5, p=0.03), although between these groups and the controls there was no difference. Contrary, there wasn't any statistically significant difference at the expression of P-Akt and P110β between the three groups (Table 1 and Figure 3).

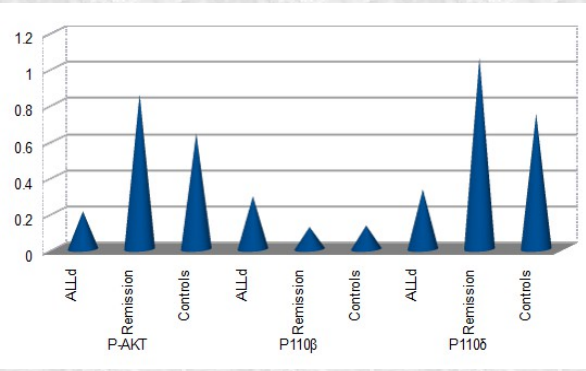

Figure 3. Graph of the medians for P-AKT, P110β and P110δ.

Conclusions

P110δ expression was estimated significant between ALL diagnoses and children in remission, but the quantity of P-Akt and P110β doesn't seem to be related with ALL.

References

1. Bhojwani D, Yang JJ, Pui CH. Biology of childhood acute lymphoblastic leukemia. Pediatr Clin North Am. 2015;62(1):47-60.

2. Jabbour E, Ottmann OG, Deininger M, Hochhaus A. Targeting the phosphoinositide 3-kinase pathway in hematologic malignancies. Haematologica. 2014;99(1):7-18.

3. Tasian SK, Teachey DT, Rheingold SR. Targeting the PI3K/mTOR pathway in pediatric hematologic malignancies. Front Oncol. 2014;16(4):108.