mRNA Expression of Three Isoforms of AKT in Different Human Cancer Cell Lines

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Abstract

The serine/threonine protein kinase Akt (PKB) participates in the PI3K/Akt signal transduction pathway, which plays an important role in the regulation of diverse cellular activities. The Akt family has three highly homologous isoforms: Akt1, Akt2 and Akt3. The three isoforms are encoded by three different genes. Research shows that elevated expression of Akt isoforms are found in different tumors. In this study, mRNA expression of Akt1, Akt2 and Akt3 were detected in six different human cancer cell lines: A549, Caco-2, K562, JMN, LAI-5S and LAI-55N. JMN, LAI-5S and LAI-55N are neuroblastoma cell lines of various malignancies. RT-PCR detected presence of all three Akt isoforms in each cell line studied. Akt2 and Akt3 exhibited differential mRNA expression while no difference in Akt1 mRNA expression was observed.

Introduction

The serine-threonine protein kinase Akt(PKB) involves in many intracellular activities such as cell proliferation, apoptosis, transcription, cell migration and glucose metabolism. It is a key downstream effector of phosphoinositide 3-kinase (PI3K). The PI3K/Akt pathway seems to be one of the most potent survival signaling pathways. Deregulated signaling through the PI3K/Akt pathway is common in many types of tumors, such as breast, prostate, and lung cancers. In cancers, aberrant activation of the PI3K/Akt pathway can be caused by genetic lesions or by tyrosine kinase receptors stimulated by growth factors. Akt is activated by a multi-step regulatory mechanism that requires both translocation to the plasma membrane and phosphorylation of two crucial amino acids (Bellacosa et al., 1988) (Figure 1.). Binding of growth factors leads to receptor activation and phosphorylation on tyrosine residues. This leads to the recruitment of PI3K to plasma membrane. Once localized to the plasma membrane, PI3K phosphorylates phosphatidylinositol-3,4-bisphosphate and generates phosphatidylinositol-3,4,5-triphosphate, which in turn binds to Akt and 3-phosphoinositide-dependent kinase-1 (PDK-1), promoting their translocation to the plasma membrane. PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene. The PTEN protein is a lipid phosphatase which can dephosphorylate phosphatidylinositol-3,4,5-triphosphate, which is the lipid product of the PI3K kinase. Thus, it antagonizes signaling through the PI3K pathway. After translocalization, Akt is phosphorlated on two amino acids and therefore activated. Activated Akt phosphorylates multiple proteins on their serine and threonine residues and carries out its function. Considering that cell proliferation, growth and apoptosis are important hallmarks of tumorigenesis, it is not surprising that Akt is one of the most frequently hyperactivated kinases in cancers.

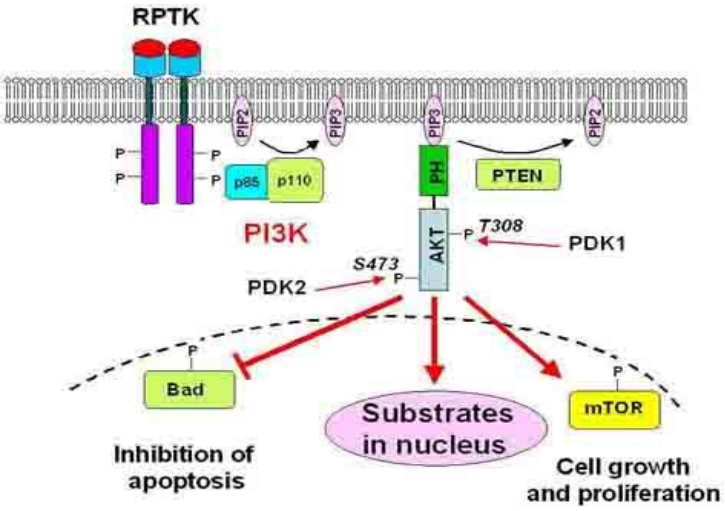

Figure 1. PI3K/Akt Signal transduction pathway

The Akt family has three closely related isoforms Akt1, Akt2 and Akt3 encoded by three different genes located on chromosome 14q32, 19q13 and 1q43. They are widely distributed in human tissues, though Akt3 expression seems to be more restricted, being primarily in brain and testis. Each isoform has 13 coding exons and 3 conserved protein domains. They are not only structurally similar, but also thought to be activated by a common mechanism. All three Akt proteins contain an N-terminal pleckstrin homology(PH) domain, a catalytic kinase domain and a C-terminal regulatory domain. The PH domain could bind specifically to phosphatidylinositol-3,4,5 -triphosphate and hence be recruited to the plasma membrane, where Akt becomes phosphorylated in the catalytic domain at threonine308(T308) by PDK-1. In addition, Akt is phosphorylated in the regulatory domain at serine473 (S473) through mechanisms not completely understood (Figure 2.). Phosphorylation of Akt at both T308 and S473 is required for full kinase activity (Bellacosa et al., 1988).

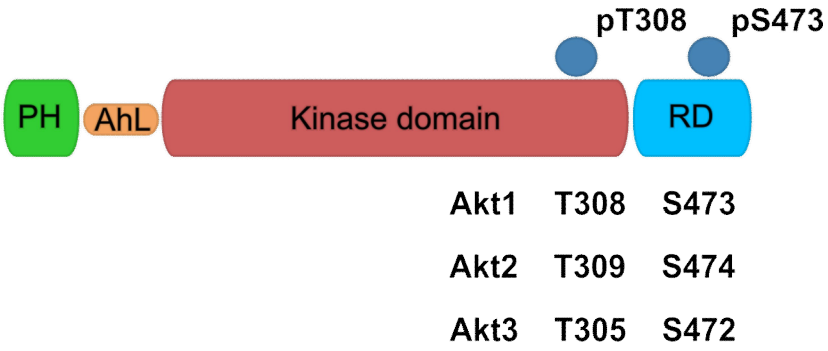

Figure 2. Schematic protein structure of Akt

Studies of Akt isoform-specific knockout mice suggest that Akt family members may have different functions. Akt1 knockout mice are smaller and have a shorter life span than wild type counterparts. Akt1-null cells display higher rates of apoptosis, indicating a critical role for Akt1 in cell survival (Chen et al., 2001). Akt2 knockout mice show insulin resistance and develop a type 2 diabetes-like phenotype, suggesting a central role for Akt2 in the glucose homeostasis (Garofalo et al., 2003). Akt3 knockout mice display impaired brain development which proposes a role for Akt3 in brain development (Tschopp et al., 2005). Although those data support the hypothesis that different cellular processes are controlled by different Akt isoforms, phenotypic analyses of double isoform knockout mice reveal some function overlap among the isoforms. All together, those studies suggest that Akt family members may have specific as well as overlapping functions.

The first evidence leading to a role of Akt in tumorigenesis was given by early studies of transforming murine virus ATK8. In human cancers, Akt deregulated activities could result from several mechanisms: inappropriate activation of the upstream partner PI3K, loss of PTEN, Akt gene amplification and Akt protein overexpression.

Overexpression of Akt isoforms are reported in different cancers. One mechanism for Akt isoform increased expresison is gene amplification. Akt1 amplification has been found in gastric cancer (Staal, 1987), Akt2 gene amplification has been found in ovarian and pancreatic cancers, with ten percent of pancreatic cancer cells showing Akt2 amplification (Cheng et al., 1996). Overexpression independent of gene amplification has also been found among different cancers. For example, increased Akt2 kinase activity was described in approximately 40% of primary ovarian cancers (Cheng et al., 1996) and Akt3 mRNA has been shown to be upregulated in certain types of melanoma (Stahl et al., 2004). Moreover, gene amplifications of Akt2 and Akt3 tend to relevant with the more aggressive tumor phenotype. Despite these important observations, to date, our understanding of the distinctive roles of Akt isoforms in tumorigenesis is still limited.

To study the relationship of Akt isoforms with cancer, we detected mRNA levels of each Akt isoform using RT-PCR in six different human cancer cell lines: A549, Caco-2, K562, JMN, LAI-5S and LAI-55N. Results reveal that all three isoforms are present in the cell lines studied. mRNA expression of Akt2 and Akt3 share similar pattern in that both show higher levels in A549 and LAI-5S cell lines. No differential expression is found in Akt1.

Material and methods

Human Cancer Cell lines

Six human cancer cell lines are used. Neuroblastoma cell lines including JMN, LAI-55N and LAI-5S are obtained from the Laboratory of Neurobiology at Fordham University. JMN is the representative of the I-phenotype, LAI-55N is the representative of the N-phenotype and LAI-5S is the representative of S-phenotype. Three neuroblastoma cell lines were cultured in DMEM/F 12 medium supplemented with 10% Fetal Bovine Serum at 37°C. Three other cell lines including A549, Caco-2 and K562 are obtained from Laboratory for Familial Dysautonomia Research. K562 were cultured in RPMI medium supplemented with 10% Fetal Bovine Serum at 37°C. A549 and Caco-2 were cultured in MEM medium supplemented with 10% Fetal Bovine Serum. EasyTides® α-33P-dATP at 10 μCi/μl or 3000Ci/mmol from PerkinElmer was used (2μl).

RNA extraction

Total RNA from each cell line was extracted and purified from the cell lysates using the RNeasy® Plus Mini Kit (QIAGEN), according to the manufacturer's instructions with some minor modifications. The RNA was eluded with 50μl of dH2O. Spectrophotometer was used to test the concentration of each RNA extraction. A 5ng/μl dilute was made from each RNA stock and used in RT-PCR.

RT-PCR

RT□PCR was performed using QIAGEN® One-Step RT□PCR Kit following the instructions. GAPDH was used as the loading control. Twenty-five nanograms of RNA were amplified in 25μl RT-PCR reactions (5μl 5×RT buffer, 1μl 10mM dNTPs, 1μl enzyme mix, 1μl 10pmol/μl forward primer, 1μl 10pmol/μl reverse primer, 2μl 5ng/μl RNA and 14μl dH2O).Temperature cycles are as follows: one cycle of 50°C for 30min and 95°C for 15min, 30 cycles of 94°C for 30sec, 58°C for 30sec, and 72°C for 30sec, then a final extension of 72°C for 7min followed by a final hold at 4°C.

Gel Electrophoresis

5μl of each RT-PCR product with 2.5μl of loading dye were seperated on 2% agarose gels (4.0 mg agarose, 200ml 1X TBE, 16.5μl ethidium bromide). The voltage of electrophoresis is 170V. Gels were visualized in BioRad UV trans-illuminator.

PCR product purification, gel extraction and sequencing

PCR poducts were purified using QIAquick® PCR Purification Kit following the manufactures instructions and sequenced in order to confirm the identity of the PCR products. Products of different Akt isoforms were cut from the gel and purified using QIAquick Gel Extraction Kit following the manufacturer's instructions. Purified PCR products were sent out for sequencing.

Primers

For each Akt gene, a pair of primers is designed using the mRNA sequence information obtained from Genbank. GAPDH is used as a loading control. Location of each primer is shown in Figure 3. Primer sequences are listed in Table 1.

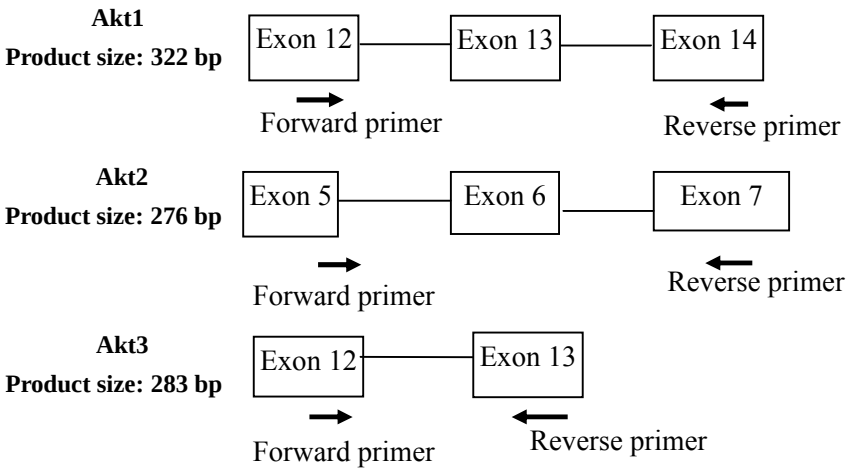

Figure 3. Primer locations of Akt1, Akt2 and Akt3

Primer	Sequence (5'→3')	Product size (bp)
GAPDH Forward	GAAGGTGAAGGTCGGAGT	226
GAPDH Reverse	GAAGATGGTGATGGGATTTC	
Akt 1 Forward	T7: TAATACGACTCACTATAGGG CCAAGGAGATCATGC	322
Akt 1 Reverse	SP6:GATTTAGGTGACACTATAG CTCCAAGCTATCGTCC	
Akt 2 Forward	T7: TAATACGACTCACTATAGGG CCATGGACTACAAGTG	276
Akt 2 Reverse	SP6:GATTTAGGTGACACTATAG TGTGAGCGACTTCATCC	
Akt 3 Forward	T7: TAATACGACTCACTATAGGG GTGCAAGGATTGTACACG	283
Akt 3 Reverse	SP6:GATTTAGGTGACACTATAG GTTGCCAGGTCATCATAACG	

Table 1. Primer sequences for Akt1, Akt2, Akt3 and GAPDH.

Results

RT-PCR using the primers (Table 1.) was conducted in three human cancer cell lines derived from different tissues. GAPDH is served as a loading control. The A549 cell line was developed through the removal and culturing of cancerous lung tissue. Caco-2 cell line is derived from a colon carcinoma. K562 cells were the first established human immortalized myelogenous leukemia line. Results (Figure 4.) show that Akt1, Akt2 and Akt3 all present in those three cell lines. mRNA levels of Akt1 seems to be similar, while both Akt2 and Akt3 exhibit highest levels in A549 cell line. In addition, Akt3 shows least mRNA expression in Caco-2 cell line. Though lower than the mRNA level shown in A549 cell line, no significant difference is exhibited in Akt2 between Caco-2 and K562 cell lines.

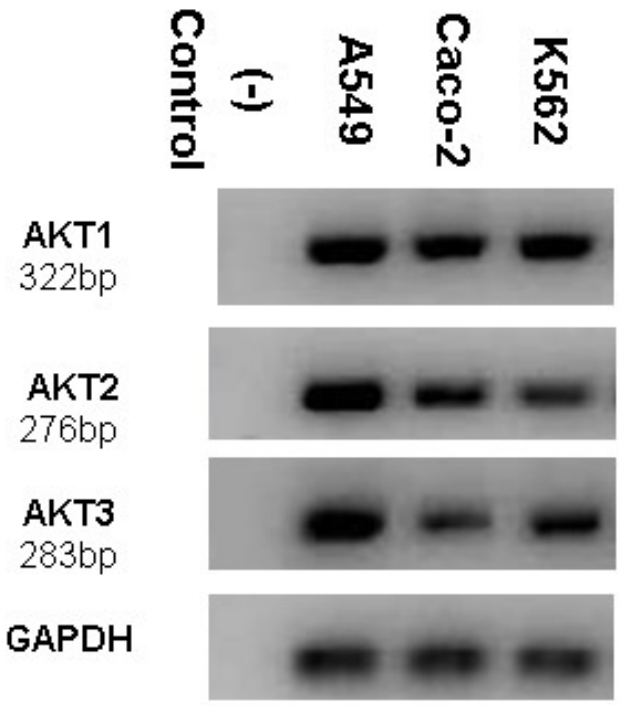

Figure 4. mRNA expression of Akt1, Akt2 and Akt3. RT-PCR was performed in A549, Caco-2 and K562 cell lines. Products are fractionated on 2% agarose gels. GAPDH is used as loading control.

mRNA expressions of Akt isoforms were also studied in three neuroblastoma cell lines: JMN, LAI5-S and LAI-55N. GAPDH is served as a loading control. The three neuroblastoma cell lines are representatives of different phenotypes and have different malignancies. JMN is the most malignant one while LAI-5S has little malignancy. Results from RT-PCR show that, again, three Akt isoforms all present in the above neuroblastoma cell lines. Though no difference is exhibited in Akt1 among the three neuroblastoma cell lines, Akt2 and Akt3 express at higher levels in LAI-5S cell line. In the JMN and LAI-55N cell lines, Akt2 and Akt3 are not differentially expressed.

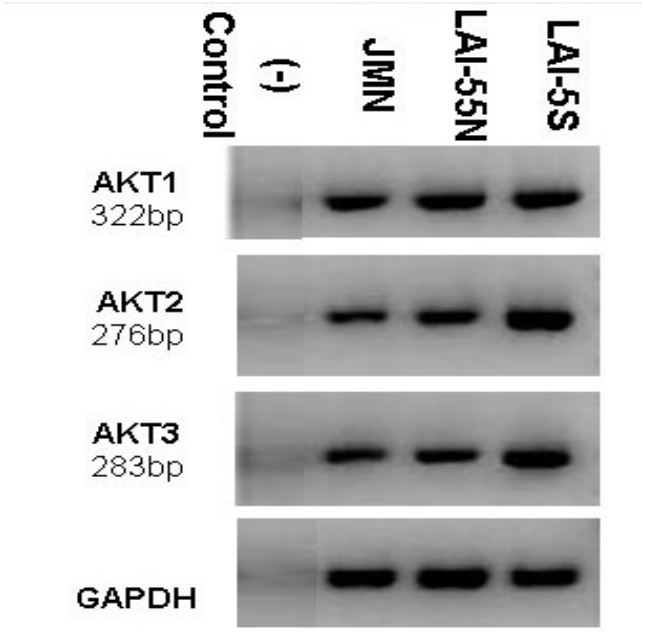

Figure 5. mRNA expression of Akt1, Akt2 and Akt3. RT-PCR was performed in neuroblastoma cell lines:JMN, LAI5-S and LAI-55N. Products are fractionated on 2% agarose gels. GAPDH is used as loading control.

Discussion

In summary, all three isoforms are expressed in the six cancer cell lines. Previous reports on Akt isoform expression in different tissues indicate that Akt1 and Akt2 are widely distributed, yet Akt3 is expressed mostly in brain and testis. However, our result shows an unrestricted expression pattern of Akt3. This may be due to the limited tissues characteristics and the increasing cancerous features of the cell lines studied. It is possible that tissue specific expression of Akt3 may be lost in cancer cells. No difference of Akt1 mRNA expression was observed in the six cell lines. mRNA expression of Akt2 and Akt3 share similar patterns. Both present at higher levels in A549 cell line and LAI-5S cell line. In fact, relevant research regarding the role of Akt in lung cancer demonstrated that specific depletion of Akt2 in A549 cells significantly reduce cancerous growth and anchorage independent growth and invasion, as compared to Akt1 depletion (Kim et al., 2010). High expression of Akt2 shown in this project is consistent with its suggested role in oncogenesis in A549 cell line. However, surprising results about Akt expression were shown in neuroblastoma cell lines. Among the three neuroblastoma cell lines, LAI-5S, the least malignant one, shows higher mRNA expression of both Akt2 and Akt3. Considering the tumorigenesis potential of Akt isoforms, why Akt2 and Akt3 show elevated mRNA level needs further study on function of Akt isoforms in neuroblastoma.

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