

# Dynamics of *Akt* Isoforms and Role of Immune Evader (*RCAS 1*) in Different Grades of Breast Cancer Tissues in Pakistani Women

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## Abstract

Breast Cancer (BC) is the leading cause of death among females. It has been documented that *Akt* isoforms and kRaS control the proliferative pathways in most cancer including BC. To address the issue of breast cancer signaling through mRNA quantification along with proteins quantifications in *Akt* pathway by microbead assay.

A total of 42 specimens were selected from a pool of samples representing nearly all Bloom's Richardson's grades tissues (Normal, Hyperplasia, Ductal in situ Carcinoma, Grade I, Grade II and Grade III). No Grade IV sample was available. Total of 8 genes (*Akt 1*, *Akt 2*, *Akt 3*, *p53*, *RCAS 1*, *Bcl 2*, *BclxL*, and *Mcl 1*) and 11 proteins in PI3K / *Akt* - mTOR pathway were assayed simultaneously using microbead BioPlex™ assay. Results on quantification of *RCAS 1* gene (Immune Evader) indicates its significance by increased quantities to help to evade the breast cancer cells to distant places to metastasize in the body. Data obtained on *Akt* isoforms (*Akt 1*, *Akt 2*, *Akt 3*) show that all three play significant role in the progression of breast cancer. Our data indicate that in advance grades (grade II and III) *Akt 3* along with *Akt 1* act as oncogenes. Here we propose that *Akt1* and *Akt 3* appear to be involved in cellular growth/proliferation and angiogenesis/invasion respectively. Bioinformatics modeling have shown that the conformation of *Akt 3* differs from *Akt 1* and *Akt 2* in PH domain that interacts to surface membrane from inner side.

Based on our data, we conceived that roles of all three *Akt* isoforms in growth, hypertrophy, epithelial mesenchymal transition (EMT), adhesion, and metastasis been highlighted. As reported in other cancers, *Akt 3* appears to be involved in the phosphorylation of VEGF and Integrin family proteins, thereby facilitating invasion and metastasis in the aggressive stages of breast cancer. We propose a model with dynamics of *Akt* and *RCAS 1* for metastasis.

## Introduction

In this part of world (Pakistan), breast cancer is the second most common cancer after lung cancer and is ranked first in women<sup>[1-4]</sup>. The current information indicates that breast cancer is most common in young women of Pakistan<sup>[5-9]</sup>. It has been substantiated that *Akt* and kRaS-MAPK are dominant proliferative pathways stimulated by activated Receptor Tyrosine kinase (RTK)<sup>[10,11]</sup>. *Akt* (also termed Protein Kinase B – PKB) works as double edged sword for cell survival as well as inhibitor of apoptosis<sup>[12,13]</sup>. It has been identified that *Akt*, especially *Akt 1* isoform is the principle oncogene<sup>[7]</sup>. More recently, three isoforms namely, *Akt 1*, *Akt 2*, *Akt 3* have been reported<sup>[1,2]</sup>. The function of each isoform has yet to be fully substantiated. So far three corresponding *Akt* genes (*Akt 1*, *Akt 2* and *Akt 3*) have been reported<sup>[14-17]</sup>. The differences in functional abnormalities observed in *Akt* null mice suggest that all isoforms have physiologically diverse roles<sup>[18]</sup>. Characterization of *Akt 1* (Ch 14q32.33), the prototype isoform, makes it the key signaling molecule in diverse cellular processes including muscle hypertrophy<sup>[19-21]</sup> and presented as a principle proliferative molecule in many types of cancer<sup>[22,23]</sup>. *Akt 2* (Ch 19q13.2) in contrast to *Akt 1*, knockout

results in mild growth deficiency with severe diabetic phenotype (insulin resistance)<sup>[23,24]</sup>. The function of *Akt 3* (Ch 1q44), a comparatively less explored isoform, is predominantly associated with brain growth and testes<sup>[20,25,26]</sup>. The *Akt* isoforms are ~480 amino acids long protein with a large number of posttranslational phosphorylation sites<sup>[10,27]</sup>. Initial records indicate that *Akt 3* is consistently detected in cancer tissues as compared to normal tissues. However, this remains controversial<sup>[28,29]</sup>. Furthermore, increase in quantities of *RCAS 1* seems to be associated with release of *Akt 3* from Golgi complex<sup>[30-32]</sup>. *RCAS 1* is over ex-

**Received date: May 7, 2017**

**Accepted date: March 8, 2018**

**Published date: March 13, 2018**

**Citation:** Sheikh, A.S., et al. Dynamics of *Akt* Isoforms and Role of Immune Evader (*RCAS 1*) in Different Grades of Breast Cancer Tissues in Pakistani Women. (2018) Int J Cancer Oncol 5(1): 16- 25.

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pressed in many patients suffering from breast carcinoma and its expression levels correlate with tumor grades as well, suggesting that it may be involved in immune escape<sup>[31,33]</sup>. *RCAS 1* is also been implicated in regulatory functions with VEGF in ovarian cell line<sup>[34,35]</sup>.

*Bcl 2* family genes / proteins maintain a critical balance between cell proliferation and apoptosis<sup>[36,37]</sup>. These antiapoptotic proteins include *Bcl 2*, *BclxL* and *Mcl 1*. These proteins have a common hydrophobic groove formed by BH 1, BH 2 and BH 3 domains<sup>[38-40]</sup>. Evidence is also available for the overexpression of some members of the *Bcl 2* family in breast cancer<sup>[36,40]</sup>. The tumorigenic potential of these proteins has been well documented in animal models in which expression of *Bcl 2* oncogene has been reported in a variety of tumors and in lymphomas<sup>[41,42]</sup>. High expression of *Bcl 2* has been observed in Estrogen Receptor (ER) and Progesterone Receptor (PR) positive breast cancer<sup>[43,44]</sup>. *BclxL* on the other hand is related to the invasion and metastasis of some solid tumors<sup>[40,45,46]</sup>. *Mcl 1* is shown to be regulated by mTOR complex for transcription and translation<sup>[47,48]</sup>. Significance of *Mcl 1* expression has been studied in a number of tumor types. It is linked to poor patient prognosis and survival<sup>[49]</sup>.

The three isoforms have been studied in melanoma<sup>[14,50,51]</sup>. In melanoma there is sharp rise in *Akt 3*<sup>[52,53]</sup>. This aspect has, however, not been studied adequately in other cancers, including breast cancer. Addition to it, little information is available on the molecular pathogenesis of *Akt 1*, *Akt 2* and *Akt 3* in various grades of cancer. Literature doesn't provide concurrent role of the three isoforms in breast cancer. Lack of this information about the dynamics of *Akt* isoforms has prompted us to examine the amplification of *Akt 1*, *Akt 2* and *Akt 3* in normal, Grade I, Grade II and Grade III tissues supported by assay of 11 phosphoproteins of *Akt* pathway. This will be first report on the dynamics of *Akt* isoforms and their associated substrates in Pakistani (Punjabi) population.

## Materials and Methods

### Specimen Collection and Processing

Total of 42 breast cancer specimens in different Bloom Richardson's grades<sup>[28]</sup> were collected from three major public and private hospitals of Lahore, Pakistan. The specimens were stored at  $-40^{\circ}\text{C}$  (Forma Scientific, USA) that includes Formalin Fixed Paraffin Embedded (FFPE) tissue blocks and Formalin Fixed (FF) tissues. The tissues were grounded to powder form in thistle and mortar with liquid nitrogen (Fine Gas (Pvt) Ltd, Lahore). Materials were taken out when ever needed on crushed ice bucket (Zellegra Eismaschinen, Germany) for further processing.

### RNA Isolation, cDNA, Primer Design and qPCR Quantification

Total RNA was extracted by LiCl – Urea method (3M LiCl, 6M Urea, 50 mM Tris (7.4), 1 mM EDTA Na<sub>2</sub>, 0.5% Sarkosyl (Serva, Germany) to preweighed tissue specimen (40-60 mg)<sup>[54]</sup>. Total of 15 – 20  $\mu\text{L}$  of 10% SDS was added to LiCl – Urea reagent to enhance homogenization with Micropestle (Bel Air Products, USA) two times for 5 minutes each. Total RNA was extracted from cells treated with Proteinase K (Vivantis, Malay-

sia) in PK buffer (10 mM Tris (8.0), 2 mM EDTA Na<sub>2</sub> (8.0), 200 mM NaCl, 0.5% SDS and 200  $\mu\text{g} / \text{mL}$  Proteinase K (20 mg/mL) for 30 minutes at  $37^{\circ}\text{C}$ . Samples were Phenol – Chloroform – Isoamyl alcohol (PCI) extracted (25:24:1) (MP Bio, USA) twice and with chloroform (Merck, Germany) once<sup>[55]</sup>. The aqueous phase was precipitated with HPLC grade 2-Propanol (BDH, UK) over night at  $-40^{\circ}\text{C}$ <sup>[56]</sup>. A second precipitation was done with  $\frac{1}{2}$  volume Ammonium acetate (5 M) and 2.5 volume of molecular grade ethanol (Merck, Germany) after pellet been dissolved in 100  $\mu\text{L}$  of RNase free water. Sample was centrifuged (Sigma, Germany) at 17 kg for 20 minutes at  $4^{\circ}\text{C}$ , pellets washed with 70% RNase free ethanol, air dried and then at  $45^{\circ}\text{C}$  for 10 minutes on heating block (T-box, Jena Analytika, Germany).

Pellets were dissolved in molecular grade water and RNA concentration was adjusted to 1  $\mu\text{g}/\mu\text{L}$  (Jena Analytika, Germany) used for cDNA synthesis by setting up a RT reaction with Maxime™ RT Random Hexamer kit (Intron Biotechnology, S. Korea) in 20  $\mu\text{L}$  volumes. The RT PCR program was written on Thermocycler (BioRad C1000 with CFX96 detector, USA) with annealing temperature of primers at  $16^{\circ}\text{C}$  (10 minutes),  $25^{\circ}\text{C}$  (10 minutes),  $37^{\circ}\text{C}$  (40 minutes) and final elongation at  $42^{\circ}\text{C}$  for 20 minutes. Reaction was denatured at  $95^{\circ}\text{C}$  for 5 minutes. Multiplex real time (rt) quantification were done with gene specific primers for *Akt 1* (AlexaFluor 647), *Akt 2* (JOE), *Akt 3* (Cy 5), *p53* (FAM) with GAPDH (ROX) as housekeeping normalization. Primers were designed from FASTA sequences uploaded on to either Light Upon eXtension (LUX™) primer design software; D – LUX primer design software (Invitrogen Corp., USA) or QuantPrime (Max Planck Institute, Germany). Labeled primers with fluorescent tags were synthesized from BioBasicInc, USA or IDT, USA. Primer sequences are given Table 1 and Table 2. All qPCRs were done by using qMaster Mix with UDG (Invitrogen, USA). Total of 2  $\mu\text{L}$  cDNA reaction, 400 nmol of each fluorescent labeled primer were used in each 20  $\mu\text{L}$  reactions. Amplification protocol was initiated with heating at  $52^{\circ}\text{C}$  (2 minutes), denaturation at  $95^{\circ}\text{C}$  (10 minutes), cycle denaturation at  $95^{\circ}\text{C}$  (20 seconds), annealing at  $58^{\circ}\text{C}$  (30 seconds), and elongation at  $72^{\circ}\text{C}$  (30 seconds). The fluorescent signal was collected for 45 cycles. The CFX manager (BioRad, USA) was used to optimize the quantified data. Each quantification reaction was done three times in separate experiments.

### Micro Bead Milliplex MAP 11-plex Assay

*Akt / mTOR* Panel (Milliplex, USA) was selected for 19 selected samples with traditional Bioplex / Luminex MAP based platform to detect simultaneously multiple protein quantities. Each sample was weighed  $\sim 50$  mg in sterile, oven dried microfuge tube for protein isolation. Assay was run according to the manufacturer manual's protocol provided with the *Akt – mTOR* panel kit. Before the processing of each sample, tissue powder was washed in 0.5 mL ice cold TBS and to pellet 150  $\mu\text{L}$  ice cold 1X Milliplex MAP Lysis Buffer containing freshly prepared 1X protease inhibitors and 1X phospho inhibitors cocktails (Serva, Germany). Gently rock the lysate for 10 - 15 minutes at  $4^{\circ}\text{C}$ . Remove particulate matter by high speed centrifugation at 15k g. Aliquot clarified supernatant and store the lysate at  $-40^{\circ}\text{C}$ . Lysate protein concentrations were determined by diluting 1:10 in PBS by Qubit™ (Invitrogen, USA) protein assay.

### Statistical Data Analysis

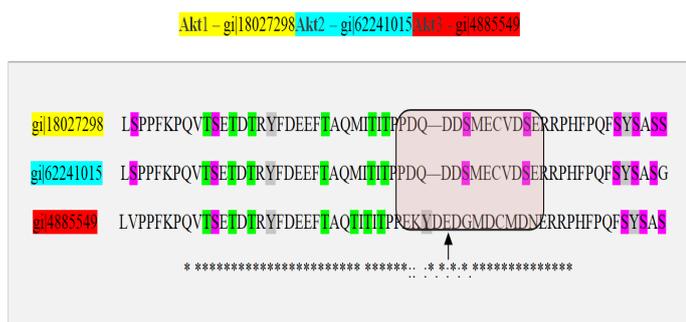
All genes and protein quantifications were statistically analyzed by SPSS 17 (SPSS, Inc. USA) using various tools in the software.

**Bioinformatics Tools:** Different bioinformatics tools were used to get 3D protein structures of different molecules which includes; *Akt 1*, *Akt 2*, *Akt 3* by loading protein FASTA Sequences on to LOMETS software (<http://zhanglab.ccmh.med.umich.edu/LOMETS>). The molecules were displayed by PyMol, Version 1.3 (2010).

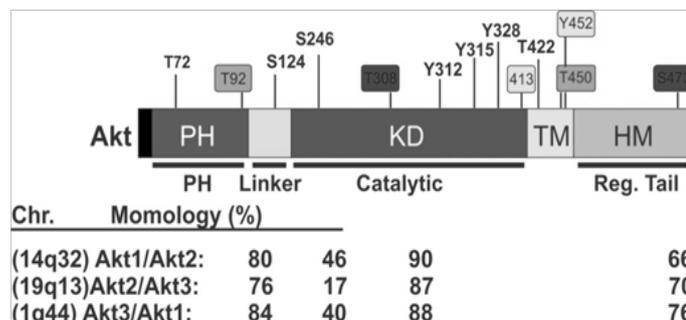
### Results

#### Physical features of isoforms

In the Figure 1a that represents the conserved domains of the three isoforms. The same figure also shows the percentage of homology between three *Akt* isoforms. Pleckstrin Homology (PH) domain has the highest homology percentage between the three isoforms<sup>[29]</sup>. The highest percentage of homology is observed between *Akt 1* and *Akt 3* (84%), followed by 80% between *Akt 1* and *Akt 2*. There is only 76% homology between *Akt 2* and *Akt 3*. Variations in other domains have also been recorded (Figure 1a).



The primary sequence was down loaded in FASTA format and protein alignment was done with MAFFT tool. Serine, threonine and tyrosine amino acids were highlighted with; S Serine T Threonine Y Tyrosine in the aligned amino acid sequence. The box represents a hyper variable region in the kinase-regulatory region where Y<sup>452</sup> is identified as unique addition. These amino acids are labeled in the LOMETS 3D prediction software. All 3D data is visualized in PyMol. The highlighted box represents the variable region in regulatory linker region of the three Akt isoforms



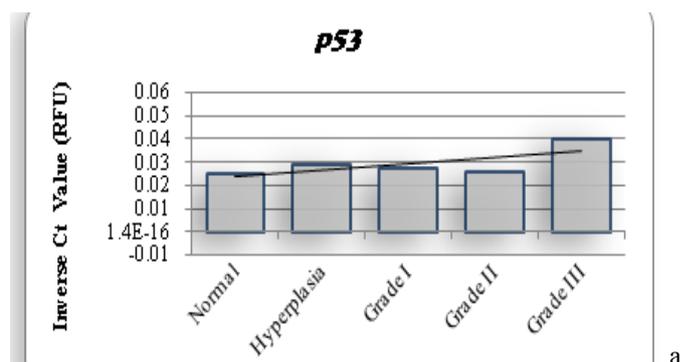
**Figure 1a:** Clustal format | Fasta format | MAFFT result.

Figure 1b shows the amino acid alignment of three isoforms ([www.ncbi.org](http://www.ncbi.org)). It has been observed that there are major variations in amino acid sequences of the three isoforms that

reside in their regulatory domain. Figure 2 shows the comparison of tertiary structure of three isoforms (<http://zhanglab.ccmh.med.umich.edu/LOMETS>). It may be seen, as expected from the major homologies between three isoforms that there is very little difference in their tertiary structure. *Akt 3*, however, shows an open loop in which Y<sup>452</sup> is available for phosphorylation. The significance of this difference is discussed elsewhere in this report. It is now known that PIP 3 mediated phosphorylation of Akts by PDK 1 in kinase domains of *Akt 1* - T<sup>308</sup>, *Akt 2* - T<sup>306</sup>, and *Akt 3* - T<sup>305</sup> and by PDK 2 in the regulatory domains of *Akt 1*-S<sup>472</sup>, *Akt 2*-S<sup>478</sup>, and *Akt 3*-S<sup>474</sup> [57]. It is also known that the phosphorylated isoforms are negatively regulated by PHILPP1 and PHLiPP2. Phosphorylated threonine in the catalytic domain in each isoform is deregulated by SHiPs<sup>[58-60]</sup>. In figure 3 (a, b, c, d) are recorded results of amplification of *Akt* isoforms in the normal, Grade I, Grade II, and Grade III tissues of breast cancer. It may be noted that *Akt 3* is conspicuously absent in the normal tissue while *Akt 1* and *Akt 2* show normal values though *Akt 2* is higher than *Akt 1*. Progressively *Akt 1* shows significant increase through Grade I, Grade II and Grade III. There is eye-catching-difference in *Akt 1* in grade I and Grade III. *Akt 3* shows highly significant increase in Grade III compared to Grade I and Grade II. It shows elevation from the normal in Grade I and Grade III. The dynamics of three isoforms obtained in the study indicate that there is a considerable variation in three isoforms during the early and later stages in breast cancer tissue. A few samples of DISC and hyperplasia were also examined (data not shown in this report). From the data obtained on *Akt* isoforms the possible conceived role of three isoforms is presented in figure 4. In normal tissue only *Akt 1* and *Akt 2* are present. However, in the progression of breast cancer, there is a major event of transformation of epithelial cells to mesenchymal cells. For this at least in breast cancer we have observed that all three isoforms play a positive role in this transformation. As the tumor progresses, further invasive and metastasis stages, *Akt 1* and *Akt 3* seem to play dominant roles in metabolism, protein synthesis, proliferation, adhesion and growth<sup>[61,62]</sup>.



**Figure 1b:** Structure and *Akt* isoform Domains. PH – Pleckstrin Homology Domain, KD – Kinase Domain, TM – Trans membrane region, HM – Hydrophobic Motif (Regulatory tail). S – Serine, T – Threonine, Y – Tyrosine (Modified from Liao and Hung, 2010).



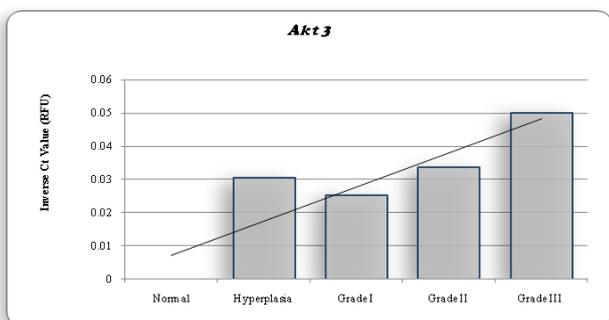
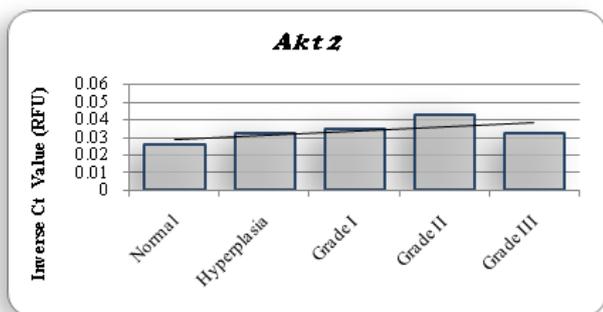
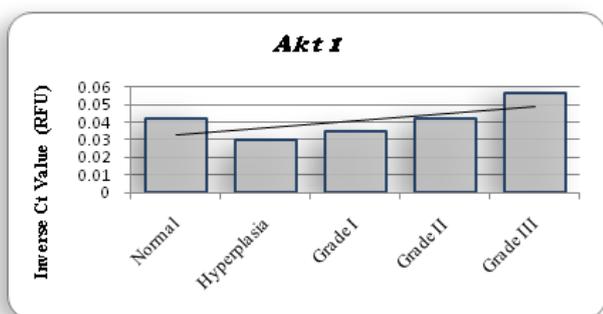
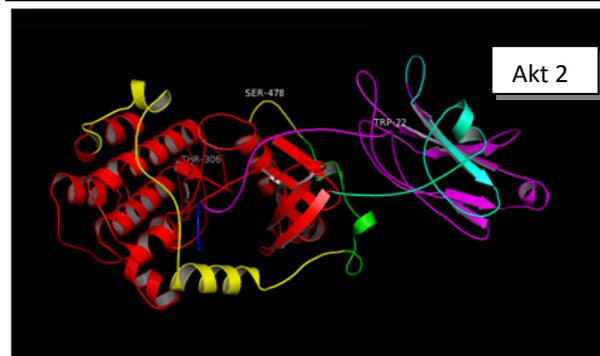


Figure 2

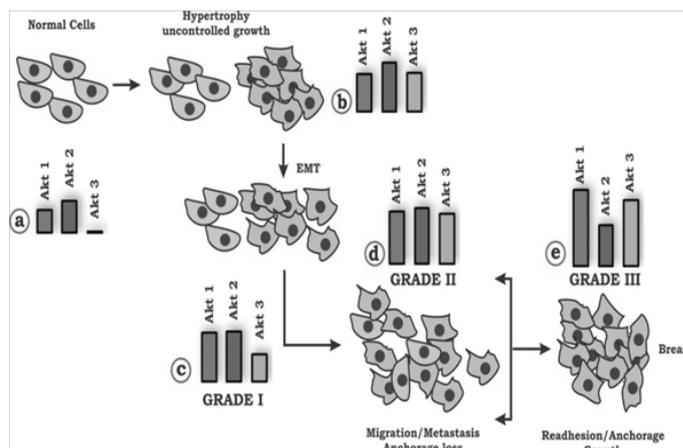


**Figure 3:** The three *Akt* Isoforms are presented the 3D structures of *Akt 1*, *Akt 2* and *Akt 3*. After downloading the sequence of each isoform from NCBI site, gene accession number NM\_005163.2 (*Akt 1*), NM\_001626.3 (*Akt 2*), NM\_181690.1 (*Akt3*). This was done to identify the possible phosphorylation sites in each isoform ([www.phosida.org](http://www.phosida.org)). It's known that *Akt 1*, 2 and 3 are phosphorylated at threonine 308, 307 and 305; at serine 474,475 and 472. These Threonine sites are phosphorylated by PDK 1. However serine sites are phosphorylated by mTOR C2, PDK 2 and ILK. These sites as may be seen in the 3D structure are exposed at the surface and are available for phosphorylation by the respective enzymes. In *Akt 3* however additional sites: Ser<sup>413</sup>, Y<sup>452</sup> is also available for phosphorylation in the regulatory domain. The possible significance of these two sites has not been reported.

b

c

d



**Figure 4:** The figure demonstrates the possible conceived role played by three isoforms of *Akt* that is *Akt 1*, *Akt 2* and *Akt 3*. It may be seen that in Normal tissue only *Akt 1* and *Akt 2* are present. However, during the progression of breast cancer, there is a major event of transformation of epithelial cells to mesenchymal cells. For this at least in breast cancer we have observed that all three isoforms play a positive role in this transformation. As the tumor progresses further into invasive and metastasis stages *Akt 1* and *Akt 3* seem to play dominant roles in metabolism, protein synthesis, proliferation, adhesion and growth.

Like the genes, 11 proteins associated with *Akt*-mTOR pathway were estimated in normal and each grade of breast cancer tissues. The measurement of 11 phosphoproteins was simultaneously accomplished with Bioplex / Luminex MAP based platform as described under materials and methods. The quantification was undertaken to find out whether these could serve as possible specific markers for various stages of breast cancer<sup>[63]</sup>. These proteins are mostly associated with metabolic requirements of proliferating cells. The estimated proteins included *Akt* (S<sup>473</sup>), mTOR (S<sup>2448</sup>), GSK3 $\alpha$  (S<sup>21</sup>), GSK3 $\beta$  (S<sup>9</sup>), p70 S6K (T<sup>412</sup>), RP S6 (S<sup>235/S236</sup>), IRS 1 (S<sup>312</sup>), PTEN (S<sup>380</sup>), TSC 2 (S<sup>939</sup>), IR (Y<sup>1162/Y1163</sup>), and IGF 1R (Y<sup>1135</sup> / Y<sup>1136</sup>). The data obtained are recorded in table 1.

**Table 1:** Table represents values of various proteins ng/mL in normal and different Grades of breast cancer tissue.

S. No	Names of Proteins	Normal	Hyperplasia	Grade I	Grade II	Grade III
1	pAkt (S <sup>473</sup> )	19.08	25.81	24.50	23.00	19.75
2	pmTOR (S <sup>2448</sup> )	19.08	20.66	20.08	18.25	18.50
3	pGSK 3 $\alpha$ (S <sup>21</sup> )	14.58	16.47	15.42	18.42	16.25
4	pGSK 3 $\beta$ (S <sup>9</sup> )	9.42	12.00	12.25	10.75	7.25
5	pp70S 6K (S <sup>412</sup> )	37.83	21.09	41.33	38.00	40.25
6	pRPS6 (S <sup>335/336</sup> )	17.67	18.97	21.50	35.75	14.00
7	pPTEN (S <sup>380</sup> )	281.08	291.78	294.17	278.58	281.25
8	pTSC2 (S <sup>939</sup> )	15.00	23.84	19.69	16.50	24.50
9	pIRS1 (S <sup>312</sup> )	8.24	12.81	10.75	11.58	12.00
10	pIR (Y <sup>1162/1163</sup> )	24.67	29.41	23.50	26.08	24.50
11	pIGF 1R (Y <sup>1135/1136</sup> )	10.58	11.72	15.58	12.75	13.00

The dynamics of RCAS 1 (Receptor associated Carcinoma Antigen on SiSo cells) like Akt isoforms was assayed in normal Grade I, Grade II and Grade III breast cancer tissues. RCAS 1 is strong indicator of immune evasion<sup>[31,34]</sup>. In all grades the relative amplification compared to normal tissue has been observed: more so in Grade II and Grade III. This indicates positive role of it in immune evasion, especially in the aggressive tissue. There is no significant difference in the amplification of any antiapoptotic genes (*Bcl 2*, *BclxL* and *Mcl 1*). This indicates that anti apoptotic role of these proteins remain unchanged. High levels of any of these *Bcl 2* family proteins in Grade I, Grade II and Grade III tissues can serve as a major marker in prognosis of breast cancer after chemotherapy.

## Discussion

In cancer, a large number of genes are known to be de-regulated<sup>[64,66]</sup>. These include the genes of cellular proliferative RaS/MAPK pathway and survival Akt pathway<sup>[66]</sup>. Several lines of evidence suggest that hyperactivation of Akt signaling along with RaS transform normal cells to cancerous cells<sup>[67-69]</sup>. In view of these findings the present study was undertaken to obtain data on the dynamics of Akt isoforms and eleven proteins associated to Akt pathway in progression of breast cancer in various stages. In addition, three antiapoptotic genes namely *Bcl 2*, *BclxL* and *Mcl 1* were quantified to assess their contribution and role in cellular proliferation. The three isoforms of Akt have shown close similarities in three dimensional structures as shown in figure 2. In spite of these similarities, specific functions have been assigned to each isoform especially in their modulations during the early and later stages of breast cancer<sup>[17,70]</sup>. Like others, we have also observed similar distribution of the three isoforms in normal, grade I, grade II and grade III breast cancer tissues. For instance, Akt 3 isoform is conspicuously absent in the normal tissue but is highly elevated in grade III tissues. This is in agreement with those reported by others<sup>[14,16,21,71-73]</sup>. Some recent reviews have discussed the functional specificity of Akt isoforms<sup>[2,74-75]</sup>. For instance, it has been suggested that Akt isoforms have relative importance in altered cells. It is substantiated that Akt 1 or Akt 2 or Akt 3 deficient mice, though viable, yet they have several deficiencies<sup>[76,77]</sup>. Another study Akt 2 knockout mice exhibited

disorder in glucose metabolism (diabetes)<sup>[78,79]</sup>. This is accompanied by insulin resistance<sup>[80]</sup>. In Akt 3 deficient mice the brain size is reduced<sup>[16,81,82]</sup>. It has also been reported that Akt 2 levels are higher in 25% of breast carcinoma<sup>[24,83]</sup>. Compared to this, Akt 3 isoform is highly elevated in advanced stages of melanoma<sup>[71,72,84]</sup>. Furthermore, high level of Akt 3 activity is demonstrated in estrogen deficient breast cancer<sup>[35,85,86]</sup>. In essence, we have concluded from our data that differential constitutive elevation of Akt isoforms is responsible for initiation as well as progression of breast cancer as reported in other cancers. These results are in agreement with those reported by others<sup>[68,71,72,83]</sup>.

A closer examination of our data reveals a relative progressive increase in Akt1, Akt 2 and Akt 3 in various grades of breast cancer tissue. This has been summarized in Normal, Grades I,II and III tissues (Figure 3). It has been documented that Akt 1 is a major isoform involved in proliferation of cancerous tissues<sup>[83,87,88]</sup>. Accordingly, the dominance of Akt 1 observed in this study is not surprising. This is further fortified by recent experimental evidence in which Akt 1 was down regulated by siRNA. This resulted in reduced proliferation<sup>[89,90]</sup>. In the same experiment, falls in the levels of Akt 1 resulted in reduced expression of Cyclin D1<sup>[85,91,92]</sup>. However, evidence to the contrary also exists. For instance, for purposes of proliferation, the role of Akt 2 cannot be minimized<sup>[79,87-95]</sup>. This was shown by enhancing the levels of Akt 2 in absence of Akt 1. In this case the proliferation was restored to the original rate of growth. It has been demonstrated that both Akt 1 and Akt 2 were required for *in vitro* and *in vivo* growth of human colon cancer cell line HCT 116 and DLD 1<sup>[96]</sup>.

In view of our data which show high levels of Akt 1 and Akt 3 and reduced levels of Akt 2 in aggressive Grade III tissue, we are inclined to interpret that these changes are related to increase in mass and metastatic transformation which is characteristic of this stage<sup>[8]</sup>. In an excellent review by Sheng *et al*<sup>[15]</sup>, it has been argued that metastasis must meet a minimum of nine requirements which include: relaxed proliferation, reduction in adhesion, a change in interaction with Extra Cellular Matrix (ECM), rapid turnover of altered fibroblasts, increase in the demand for nutrients and blood supply (angiogenesis), and focus of proliferation on metastatic sites followed by implantation<sup>[97,98]</sup>. It appears that in our case, based on quantitative gene analysis of 42 samples, the above conditions of metastasis are fulfilled primarily by Akt 1 and Akt 3 isoforms<sup>[99]</sup>.

Though our data properly exhibits the dynamics of Akt isoforms in various grades of breast cancer tissue, yet it has to be explained whether in our case the increase of mRNA is due to; a) increased turnover of mRNA, b) genetic modifications, or c) gene amplification. It has been reported that genetic modifications are very rare in Akt<sup>[16]</sup>. Similarly, gene amplification of Akt has not been reported in breast cancer<sup>[20,100]</sup>. The differential elevation of mRNA of three isoforms leads to the conclusion that mRNA of each isoform is separately regulated. Furthermore, we have examined conformational differences in the three Akt isoforms using bioinformatics tools described under materials and methods. Interestingly enough, it has been reported that transplantation of PH domain of Akt 2 onto Akt 1 didn't restore the function of proliferation characteristic of Akt 1<sup>[76,94]</sup>. We were particularly interested in identifying differences in 3D structure of PH domain for each isoform along with its phosphorylation

sites<sup>[101,102]</sup>. 3D structures of the three isoforms, shown in figure 2, have been constructed using LOMETS (<http://zhanglab.cmb.med.umich.edu/LOMETS>). It may be seen in Figure 2 that in addition to putative phosphorylation sites in the three isoforms, additional phosphorylation sites are available in *Akt 3*. The new sites of phosphorylation are S<sup>413</sup> and Y<sup>452</sup>. Whether these sites are actually phosphorylated in the signaling process remains to be determined. It is interesting to note, as is evident from our data that in Grade III along with *Akt 1* and *Akt 3*, *kRaS* is also elevated. This situation has been reported in literature in various types of cancer<sup>[35,103,104]</sup>. A model of *Akt* isoform dynamics has been reported earlier. The model is based on the modulation of only *Akt 1* and *Akt 2*. In this report the model presents elevated *Akt1* with depressed *Akt 2*. In EMT the situation is reversed and remains so in the metastatic stage<sup>[76,105]</sup>. In the data we are reporting in breast cancer patients in Pakistani females, we have observed the elevation of *Akt 1* and *Akt 3* in Grade III tissues. It is, however, important to point out that *Akt 3* is not detected in the normal tissue (Figure 3). On these bases we can suggest that elevation in *Akt 1* and *Akt 3* in Grade III tissues can be taken as biomarkers for aggressive stages of breast cancer. This calls for use of specific inhibitors of these two Akt isoforms for the treatment of cancer<sup>[27,106]</sup>.

In the Pakistani population we are reporting for the first time that *Akt 3* isoform along with *kRaS* play a dominant role in the aggressive stage of breast cancer. It is also of interest that in this population *Akt 2* is the first isoform to be elevated in hyperplasia. This is followed by elevation of *Akt 1*, *Akt 2* and *Akt 3* in subsequent stages (Figure 3 a, 3b, 3c).

In view of the well-known immunosuppressive role of *RCAS 1*<sup>[33,107-109]</sup> we were interested to study modulations of *RCAS 1* because of its relationship with *Akt 3* in various grades of breast cancer tissue. It has been documented that in tumor cells *RCAS 1* expression plays an important role in evading the cancer cells from the immune system surveillance. This promotes progression, invasion and metastasis<sup>[27,34]</sup>. The antigen has been used as a prognostic marker in Normal, Hyperplastic and malignant uterine endometrium<sup>[3,109]</sup>. It has been identified as a possible factor in endocrine-immune interaction in breast cancer<sup>[33,110]</sup>. Elevated levels of this antigen have been reported in hepatocellular carcinoma<sup>[31]</sup>, gastrointestinal tract, non-small cell lung carcinoma, gall bladder, breast, ovarian, endometrial and cervical cancers<sup>[33,111]</sup>. In our data presented in Figures 3f show the increase of *RCAS 1* in Hyperplasia, Grade I, Grade II and Grade III tissues. This increase is directly related to high levels of *Akt 3*<sup>[84]</sup>. The antigen is also listed as EBAG 9 is known to bind estrogen receptor<sup>[112]</sup> thus activation of estrogen binding receptor. Gene suppression at transcription level and of immune system with MHC class 1 molecule on surface of tumor cells signifies various mechanisms that are in place in the cell<sup>[34,113]</sup>. In view of this information it appears that elevated levels of *RCAS 1* in company with *Akt 3* promote immune cell evasion, promotes survival, boost invasion and metastasis<sup>[108,114]</sup>.

Similar conclusion could also be drawn with references to the levels of anti-apoptotic genes: *Bcl 2*, *BclxL* and *Mcl 1* (Figures 3g, 3h, 3i). Our data also indicate that quantification of *Bcl 2* family genes along with *RCAS 1* could also be used as good prognostic markers as seen in prostate cancer<sup>[40,115,116]</sup>. Such a prognostic study must be accompanied by determining the lev-

els of *Akt 3* and anti apoptotic genes in the same tissue. This relationship of *RCAS 1* with antiapoptotic genes and *Akt 3* is being reported for the first time in Pakistani (Punjabi) population.

The proteins that were assayed for normal and different grades of breast cancer tissues are listed in table 1. Little information is available about the modulations of *Akt* and its substrate proteins in various grades of breast cancer. In our hands *pAkt*, *pGSK 3β*, *pTSC 2*, *pIRS 1* and *pIR* can be conveniently used as biomarkers for Grade I and Grade II. However, we propose that in Grade III, *pTSC 2*, *pIRS 1* and *pIGF 1R* quantification may serve as useful biomarkers. The proteins which are depressed in this grade include *pGSK 3β* and *pRPS6*. Comparable analytical data on these proteins in breast cancer tissue on various grades is not available in literature and are being reported for the first time. This could promote the process of grading breast cancer combined with other pathological information on Bloom-Richardson system<sup>[28]</sup>. It is of great interest to note that simultaneous analysis of gene reported in this study along with protein analysis could be of help in selecting future inhibitors for *Akt* and its substrates. The observation that levels of *pAkt* as well as *pGSK 3α*, *pTSC 2*, *pIRS 1* and *pIGF 1R* are lowest in normal tissues (Table 1) may be of help in comparing of these proteins with their levels in various grades of cancerous tissues. *pPTEN* does not show any variation in the normal tissue as compared to its values in all other grades of cancer. *pPTEN* in active form dephosphorylates the PIP 3K stimulated phosphorylation of inositol at carbon 3<sup>[117,118]</sup>. The sustained levels of *pPTEN* in all tissue grades indicate its ineffectiveness to dephosphorylate PIP 3. We are therefore; suggest that elevated *PTEN* in the normal tissue is a mutated form of this gene (Data not shown). This has been previously reported in several studies<sup>[58,63,119-121]</sup>.

The quantification of the proteins reported here, as expected, provided interesting information with regard to *pIRS 1* and *pIGF 1R*. Insulin is the ligand for the two receptors. These two proteins are elevated in all grades of breast cancer tissue<sup>[122]</sup>. This is indicative of increased glucose metabolism (energy) as is required during growth, proliferation and metastasis<sup>[23]</sup>. It has been previously reported that *GSK 3α* and *GSK 3β* were upregulated in normal prostate and in other cancers<sup>[123]</sup>. However, in breast cancer *GSK 3β* increases in Grade I and both *GSK 3α* and *GSK 3β* increase in Grade II. This is again indicative of the availability of energy through glucose metabolism<sup>[67,68,124]</sup>.

We have estimated total mTOR complex phosphorylated at S<sup>248</sup>. mTOR C2 phosphorylates *Akt* at S<sup>472</sup> after its activation by *pRheb*<sup>[79,106,110]</sup>. Other substrates of mTOR complex include *STAT3*, *S6K1 (p70)*, *4E-BP1* and *ULK1/ATG13*<sup>[94,125]</sup>. In this way it promotes growth, proliferation, survival, angiogenesis and metabolic activities in various cancers<sup>[126,127]</sup>. Like others, we have observed increased levels of phospho *p70 S6 kinase* and *pRPS6*. Alternatively, *RPS6* is also phosphorylated by *p90 (RSK)*. In that case it inhibits the selective transcription of various other genes<sup>[94,128]</sup> making significant changes in normal cell signaling cascades. This is more evident from phosphorylated *RPS6* that promotes growth factors and tumor promoting agents<sup>[128]</sup>.

## Conclusion

In essence, our genomic and proteomic data provide substantial evidence that *Akt 1* and *Akt 3* provide signals for proliferation of tumor (size) and invasion – angiogenesis. Furthermore, the elevated levels of pIR, pIGF 1R, pIRS 1 in glucose metabolism accompanied by phosphorylation of GSK 3 $\alpha$  and GSK 3 $\beta$  provide higher energy source for growth, survival, proliferation and angiogenesis. The subtle dynamics of genes and proteins reported in this study may be of some help in selection of therapeutic molecules for breast cancer.

**Acknowledgement:** I thank University of Lahore, Lahore 54000 for providing financial support for Milliplex Akt-mTOR kit. I also acknowledge expert statistical work by MsRabailAlam, IMBB, University of Lahore for statistical layout / analysis and MsSumairaShaheen, CRiMM, University of Lahore for critical manuscript evaluation / formatting.

## References

- Hanif, M., Zaidi, P., Kamal, S., et al. Institutional based Cancer incidence in a local population in Pakistan: nine year data analysis. (2009) *Asian Pac J Cancer Prev* 10(2): 227-230.  
Pubmed | Crossref | Others
- Bhurgri, Y., Bhurgri, A., Nishter, S., et al. Pakistan country profile of cancer and cancer control 1995-2004. (2006) *J Pak Med Assoc* 56(3): 124-130.  
Pubmed | Crossref | Others
- Mamoon, N., Sharif, M.A., Mushtaq, S., et al. Breast carcinoma over three decades in northern Pakistan? Are we getting anywhere? (2009) *J PMA* 59(12): 835-838.  
Pubmed | Crossref | Others
- DeSantis, C., Siegel, R., Bandi, P., et al. Breast Cancer Statistics, 2011. (2011) *CA Cancer J Clin* 61(6): 409-418.  
Pubmed | Crossref | Others
- Women Health Problems. Breast Cancer. 2009.  
Pubmed | Crossref | Others
- Fatima, N., Zaman, M.U., Maqbool, A., et al. Lower incidence but more aggressive behavior of right sided breast cancer in Pakistani women: does right deserve more respect?. (2013) *Asian Pac J Cancer Prev* 14(1): 43-45.  
Pubmed | Crossref | Others
- Parveen, S., Sarwar, G., Khuwaja, M. Carcinoma of breast, pattern and presentation in developing countries. (2011) *Pak J Surg* 27(4): 246-249.  
Pubmed | Crossref | Others
- Cooper, C.L., Karim, R.Z., Selinger, C., et al. Molecular alterations in metaplastic breast carcinoma. (2013) *J ClinPathol* 66(6): 522-528.  
Pubmed | Crossref | Others
- Mamoon, N., Hussan, U., Mushtaq, S. Breast carcinoma in young women age 30 or less in northern Pakistan – the armed forces institute of Pathology experience. (2009) *Asian Pac J Cancer Prev* 10(6): 1079-1082.  
Pubmed | Crossref | Others
- Carnero, A. The PKB/Akt pathway in cancer. (2010) *Cur Pharmaceut Design* 16(1): 34-44.  
Pubmed | Crossref | Others
- Lemmon, M.A., Schlessinger, J. Cell signaling by receptor tyrosine kinases. (2010) *Cell* 141(7): 1117-1134.  
Pubmed | Crossref | Others
- Dillon, R.L., Muller, W.J. Distinct biological roles for the Akt family in mammary tumor progression. (2010) *Cancer Res* 70(11): 4260-4264.  
Pubmed | Crossref | Others
- Diez, H., Garrido, J.J., Wandosell, F. Specific roles of Akt isoforms in apoptosis and axon growth regulation in neurons. (2012) *PLoS One* 7(4): e32715.  
Pubmed | Crossref | Others
- Bellacosa, A., Testa, J.R., Larue, L. A portrait of Akt kinases: human cancer and animal models depict a family with strong individualities. (2004) *Cancer Biol Ther* 3(3): 268-275.  
Pubmed | Crossref | Others
- Sheng, S., Qiao, M., Pardee, A.B. Metastasis and AKT activation. (2009) *J Cell Physiol* 218(3): 451-454.  
Pubmed | Crossref | Others
- Bellacosa, A., Larue, L. PI3K/Akt Pathway and the Epithelial-Mesenchymal Transition. (2010) Springer Sci, USA.  
Pubmed | Crossref | Others
- Hemmings, B.A., Restuccia, D.F. PI3K-PKB / Akt pathway. (2012) *Cold Spring Harbor Perspect Biol* 4(9): a011189.  
Pubmed | Crossref | Others
- Hutchinson, J., Jin, J., Cardiff, R.D., et al. Activation of Akt (protein kinase B), in mammary epithelium provides a critical cell survival signal required for tumor progression. (2001) *Mol Cellular Biol* 21(6): 2203-2212.  
Pubmed | Crossref | Others
- Peng, Z., Weber, J.C., Han, Z. Dichotomy effects of Akt signalling in breast cancer. (2012) *Mol Cancer* 11: 61.  
Pubmed | Crossref | Others
- Brugge, J.S., Hung, M-C., Mills, G.B. A New Mutational Aktivation in the PI3K Pathway. (2007) *Cancer Cell* 12(2): 104-107.  
Pubmed | Crossref | Others
- Calvo, M.B., Fernandez, V.B., Villaamil, V.M., et al. Biology of BMP signalling and cancer. (2009) *Clin Transl Oncol* 11(3): 126-137.  
Pubmed | Crossref | Others
- Davies, M.A. Regulation, role, and targeting of Akt in cancer. (2011) *J Clin Oncol* 29(35): 4715-4717.  
Pubmed | Crossref | Others
- Cairns, R.A., Harris, I.S., Mak, T.W. Regulation of cancer cell metabolism. (2011) *Nat Rev Cancer* 11(2): 85-95.  
Pubmed | Crossref | Others
- Sung, J.S., Park, K.H., Kim, S.T., et al. Discovery and evaluation of polymorphisms in the Akt 2 and Akt 3 promoter regions for risk of Korean lung cancer. (2012) *Genomics Inform* 10(3): 167-174.  
Pubmed | Crossref | Others
- Do, H., Salemi, R., Murone, C., et al. Rarity of Akt 1 and Akt 3 E17K mutations in squamous cell carcinoma of lung. (2010) *Cell Cycle* 9(21): 4411-4412.  
Pubmed | Crossref | Others
- Brunelle, J.K., Letai, A. Control of mitochondrial apoptosis by the Bcl 2 family. (2009) *J Cell Sci* 122(4): 437-441.  
Pubmed | Crossref | Others
- Chin, Y.R., Toker, A. Akt isoform specific signaling in breast cancer: Uncovering an anti-migratory role for paladin. (2011) *Cell Adh Migr* 5(3): 211-214.  
Pubmed | Crossref | Others
- Bloom, H.J.G., Richardson, W.W. Histological grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 15 years. (1957) *Br J Cancer* 11(3): 359.  
Pubmed | Crossref | Others
- Carpten, J.D., Faber, A.L., Horn, C., et al. A transforming mutation in the pleckstrin homology domain of Akt 1 in cancer. (2007) *Nature* 448(7152): 439-444.  
Pubmed | Crossref | Others
- Migita, T., Inoue, S. Implications of the Golgi apparatus in prostate cancer. (2012) *Intl J Biochem Cell Biol* 44(11): 1872-1876.  
Pubmed | Crossref | Others
- Jozwicki, W., Windorbska, W., Brozyna, A.A., et al. The analysis of receptor-binding cancer antigen expressed on SiSo cells (RCAS 1); immunoreactivity within the microenvironment of the ovarian cancer

- lesion relative to the applied therapeutic strategy. (2011) *Cell Tissue Res* 345(3): 405-414.  
[Pubmed](#) | [Crossref](#) | [Others](#)
32. Grabinski, N., Bartkowiak, K., Grupp, K., et al. Distinct functional roles of Akt isoforms for proliferation, survival, migration and EGF-mediated signalling in lung cancer derived disseminated tumor cells. (2011) *Cell Signal* 23(12): 1952-1960.  
[Pubmed](#) | [Crossref](#) | [Others](#)
33. Sonoda, K. RCAS 1 is a promising therapeutic target against cancer: its multifunctional bioactivities and clinical significance. (2012) *Expert Rev Obst Gynaecol* 7(3): 261-267.  
[Pubmed](#) | [Crossref](#) | [Others](#)
34. Nishinakagawa, T., Fujii, S., Nozaki, T., et al. Analysis of cell cycle arrest and apoptosis induced by RCAS 1 expression. (2010) *Int J Mol Med* 25(5):717-722.  
[Pubmed](#) | [Crossref](#) | [Others](#)
35. Hanrahan, A.J., Schultz, N., Westfal, M.L., et al. Genomic complexity and Akt dependence in serous ovarian cancer. (2012) *Cancer Discov* 2(1): 56-67.  
[Pubmed](#) | [Crossref](#) | [Others](#)
36. Chipuk, J.E., Moldoveanu, T., Llambi, F., et al. The Bcl 2 family reunion. (2010) *Mol Cell* 37(3): 299-310.  
[Pubmed](#) | [Crossref](#) | [Others](#)
37. Renault, T.T., Chipuk, J.E. Getting away with murder: how does the Bcl 2 family of proteins kill with immunity? (2013) *Ann NY Acad Sci* 1285: 59-79.  
[Pubmed](#) | [Crossref](#) | [Others](#)
38. Bolesta, E., Pfannenstiel, L.W., Demelash, A., et al. Inhibition of Mcl 1 promotes senescence in cancer cells: implications for preventing tumor growth and chemotherapy resistance. (2012) *Mol Cell Biol* 32(10): 1879-1892.  
[Pubmed](#) | [Crossref](#) | [Others](#)
39. Kazi, A., Sun, J., Doi, K., et al. The BH3 alpha-helical mimic BH3-M6 disrupts BclxL, Bcl-2, and Mcl 1 protein-protein interactions with Bax, Bak, Bad, or Bim and induces apoptosis in a Bax- and Bim-dependent manner. (2011) *J Biol Chem* 286(11): 9382-9392.  
[Pubmed](#) | [Crossref](#) | [Others](#)
40. Dewson, G., Kluck, R.M. Bcl 2 family regulated apoptosis in health and disease. (2010) *Cell Health Cytoskeleton* 2010: 9-22.  
[Pubmed](#) | [Crossref](#) | [Others](#)
41. Flusberg, D.A., Numaguchi, Y., Ingber, D.E. Cooperative Control of Akt phosphorylation, Bcl 2 expression, and apoptosis by cytoskeletal microfilaments and microtubules in capillary endothelial Cells. (2001) *Mol Biol Cell* 12(10): 3087-3094.  
[Pubmed](#) | [Crossref](#) | [Others](#)
42. Okaro, A.C., Deery, A.R., Hutchins, R.R. et al. The expression of antiapoptotic proteins Bcl 2, BclxL, and Mcl 1 in benign, dysplastic, and malignant biliary epithelium. (2001) *J Clin Pathol* 54(12): 927-932.  
[Pubmed](#) | [Crossref](#) | [Others](#)
43. Dalafave, D., Presto, G. Inhibition of antiapoptotic BclxL, Bcl 2, and Mcl 1 proteins by small molecule mimetics. (2010) *Cancer Inform* 9: 169-177.  
[Pubmed](#) | [Crossref](#) | [Others](#)
44. Kallel-Bayouhdh, I., Hassen, H.B., Khabir, A., et al. Bcl 2 expression and triple negative profile in breast carcinoma. (2011) *Medical Oncol* 28(Suppl 1): S55-S61.  
[Pubmed](#) | [Crossref](#) | [Others](#)
45. Shamas-Din, A., Kale, J., Leber, B., et al. Mechanisms of action of Bcl 2 family proteins. (2013) *Cold Spring Harbor Perspect Biol* 5(4): a008714.  
[Pubmed](#) | [Crossref](#) | [Others](#)
46. Huang, J., Manning, B.D. A complex interplay between Akt, TSC2, and the two mTOR complexes. (2009) *Biochem Soc Trans* 37(1): 217-222.  
[Pubmed](#) | [Crossref](#) | [Others](#)
47. Colo, J.L., Macintyre, A.N., et al. Akt dependent glucose metabolism promotes Mcl 1 synthesis to maintain cell survival and resistance to Bcl 2 inhibition. (2011) *Cancer Res* 71(15): 5204-5213.  
[Pubmed](#) | [Crossref](#) | [Others](#)
48. Gupta, M., Hendrickson, A.E., et al. Dual mTORC1 / mTORC2 inhibition diminishes Akt activation and induces Puma dependent apoptosis in lymphoid malignancies. (2012) *Blood* 119(2): 476-487.  
[Pubmed](#) | [Crossref](#) | [Others](#)
49. O'Driscoll, L., Cronin, D., et al. Expression and prognostic relevance of Mcl 1 in breast cancer. (2004) *Anticancer Res* 24(2A): 473-482.  
[Pubmed](#) | [Crossref](#) | [Others](#)
50. Altomare, D.A., Testa, J.R. Perturbations of the Akt signaling pathway in human cancer. (2005) *Oncogene* 24(50): 7455-7464.  
[Pubmed](#) | [Crossref](#) | [Others](#)
51. Shao, Y., Aplin, A.E. Akt 3 - mediated resistance to apoptosis in BRAf-targeted melanoma cells. (2010) *Cancer Res* 70(16): 6670-6681.  
[Pubmed](#) | [Crossref](#) | [Others](#)
52. Kirkegaard, T., Edwards, J., et al. Molecular alterations in Akt 1, Akt 2 and Akt 3 detected in breast and prostatic cancer by FISH. (2010) *Histopathol* 56(2): 203-211.  
[Pubmed](#) | [Crossref](#) | [Others](#)
53. Farrell, R.E. *RNA Methodologies*. (2010) Academic Press USA.  
[Pubmed](#) | [Crossref](#) | [Others](#)
54. Farragher, S.M., Tanney, A., et al. RNA expression analysis from formalin fixed paraffin embedded tissues. (2008) *Histochem Cell Biol* 130(3): 435-445.  
[Pubmed](#) | [Crossref](#) | [Others](#)
55. Dey-Guha, I., Wolfer, A., Yeh, A.C., et al. Asymmetric cancer cell division regulated by Akt. (2011) *Proc Natl Acad Sci* 108(31): 12845-12850.  
[Pubmed](#) | [Crossref](#) | [Others](#)
56. Chua, B.T., Gallego-Ortega, D., Ramirez-de-Molina, A., et al. Regulation of Akt (S473) phosphorylation by choline kinase in breast carcinoma cells. (2009) *Mol Cancer* 8: 131.  
[Pubmed](#) | [Crossref](#) | [Others](#)
57. Novak, B., Kapuy, O., Domingo-Sananes, M.R., et al. Regulated protein kinases and phosphatases in cell cycle decisions. (2010) *Cur Opin Cell Biol* 22(6): 801-808.  
[Pubmed](#) | [Crossref](#) | [Others](#)
58. Chan, T.O., Zhang, J., Rodeck, U., et al. Resistance of Akt kinases to dephosphorylation through ATP dependent conformational plasticity. (2011) *Proc Natl Acad Sci* 108(46): 1120-1127.  
[Pubmed](#) | [Crossref](#) | [Others](#)
59. Sun, H., Wang, Y. Novel Serine / Threonine protein phosphatases in cell death regulation. (2012) *Physiol* 27(1): 43-52.  
[Pubmed](#) | [Crossref](#) | [Others](#)
60. Haslinger, P., Haider, S., Sonderegger, S., et al. Akt isoforms 1 and 3 regulate basal and epidermal growth factor-stimulated SGHPL-5 trophoblast cell migration in humans. (2013) *Biol Reprod* 88(3): 54.  
[Pubmed](#) | [Crossref](#) | [Others](#)
61. Liang, Y., Liu, J., Feng, Z. The regulation of cellular metabolism by tumor suppressor p53. (2013) *Cell Biosci* 3(1): 9.  
[Pubmed](#) | [Crossref](#) | [Others](#)
62. McCrea, H.J., De Camilli, P. Mutations in Phosphoinositide Metabolizing Enzymes and Human Disease. (2009) *Physiol* 24(1): 8-16.  
[Pubmed](#) | [Crossref](#) | [Others](#)
63. Agathocleous, M., Harris, W.A. Metabolism in physiological cell proliferation and differentiation. (2013) *Trends Cell Biol* 23(10): 484-492.  
[Pubmed](#) | [Crossref](#) | [Others](#)
64. Brognard, J., Hunter, T. Protein kinase signaling networks in cancer. (2011) *Curr Opin Genet Dev* 21(1): 4-11.  
[Pubmed](#) | [Crossref](#) | [Others](#)
65. Castellano, E., Downward, J. RAS interaction with PI3K: more than just another effector pathway. (2011) *Genes Cancer* 2(3): 261-274.  
[Pubmed](#) | [Crossref](#) | [Others](#)

66. Cecconi, S., Mauro, A., Cellini, V., et al. The role of Akt signalling in the mammalian ovary. (2012) *Intl J Develop Biol* 56(10-12): 809-817.  
PubMed | Crossref | Others
67. Cristiano, B.E., Chan, J.C., Hannan, K.M., et al. A specific role for Akt 3 in the genesis of ovarian cancer through modulation of G2 – M phase transition. (2006) *Cancer Res* 66(24): 11718-11725.  
PubMed | Crossref | Others
68. Denayer, E., de Ravel, T., Legius, E. Clinical and molecular aspects of RAS related disorders. (2008) *J Med Genet* 45(11): 695-703.  
PubMed | Crossref | Others
69. Virtakoivu, R., Pellinen, T., Rantala, J.K., et al. Distinct roles of Akt isoforms in regulating  $\beta$ 1-integrin activity, migration, and invasion in prostate cancer. (2012) *Mol Biol Cell* 23(17): 3357-3369.  
PubMed | Crossref | Others
70. Xu, N., Lao, Y., Zhang, Y., et al. Akt: a double-edged sword in cell proliferation and genome stability. (2012) *J Oncol* 2012: 15.  
PubMed | Crossref | Others
71. Wickendon, J.A., Watson, C.J. Key signalling nodes in mammary gland development and cancer, signalling downstream of PI3 kinase in mammary epithelium: a play in 3 Akts. (2010) *Cancer Res* 12(2): 202.  
PubMed | Crossref | Others
72. Gonzalez, E., McGraw, T.E. The Akt kinases: isoform specificity in metabolism and cancer. (2009) *Cell Cycle* 8(16): 2502-2508.  
PubMed | Crossref | Others
73. Fortier, A.M., Asselin, E., Cadrin, M. Functional specificity of Akt isoforms in cancer progression. (2011) *Bio Mol Concepts* 2(1-2): 1-11.  
PubMed | Crossref | Others
74. Toker, A. Achieving specificity in Akt signaling in cancer. (2012) *Adv Biol Regul* 52(1): 78-87.  
PubMed | Crossref | Others
75. Fortier, A.M., Van, Themsche, C., Asselin, E., et al. Akt isoforms regulate intermediate filament protein levels in epithelial carcinoma cells. (2010) *FEBS Lett* 584(5): 984-988.  
PubMed | Crossref | Others
76. Yang, W-L., Wu, C-Y., Wu, J., et al. Regulation of Akt signaling activation by ubiquitination. (2010) *Cell Cycle* 9(3): 487-497.  
PubMed | Crossref | Others
77. Buzzi, F., Xu, L., Zuellig, R.A., et al. Differential effects of protein kinase B/Akt isoforms on glucose homeostasis and islet mass. (2010) *Mol Cell Biol* 30(3): 601-612.  
PubMed | Crossref | Others
78. Chau, N.M., Ashcroft, M. Akt 2: a role in breast cancer metastasis. (2004) *Br Cancer Res* 6(1): 55-57.  
PubMed | Crossref | Others
79. Gonzalez, E., McGraw, T.E. Insulin modulated Akt subcellular localization determines Akt isoform specific signaling. (2009) *Proc Natl Acad Sci U S A* 106(17): 7004-7009.  
PubMed | Crossref | Others
80. Chen, Y.Y., Li, C.F., Yeh, C.H., et al. Interleukin-19 in breast cancer. (2013) *Clinical Develop Immunol* 2013: 9.  
PubMed | Crossref | Others
81. Toker, A. Achieving specificity in Akt signaling in cancer. (2012) *Adv Biol Regul* 52(1): 78-87.  
PubMed | Crossref | Others
82. Duan, W. mRNA expression of three isoforms of Akt in different human cancer cell lines. (2011) *Biotech*. PubMed | Crossref | Others
83. Liby, T.A., Spyropoulos, P., Buff, L.H., et al. Akt 3 controls vascular endothelial growth factor secretion and angiogenesis in ovarian cancer cells. (2012) *Int J Cancer* 130(3): 532-543.  
PubMed | Crossref | Others
84. Liao, Y., Hung, M-C. Physiological regulation of Akt activity and stability. (2010) *Am J Transl Res* 2(1): 19-42.  
PubMed | Crossref | Others
85. Nakatani, K., Thompson, D.A., Barthel, A. Upregulation of Akt 3 in estrogen receptor deficient breast cancers and androgen-independent prostate cancer lines. (1999) *J Biol Chem* 274(31): 21528-21532.  
PubMed | Crossref | Others
86. Lee, R.S., House, C.M., Cristiano, B.E., et al. Relative expression levels rather than specific activity plays the major role in determining in vivo Akt isoform substrate specificity. (2011) *Enzyme Res* 2011: 720985.  
PubMed | Crossref | Others
87. Devaney, J.M., Gordish-Dressman, H., Harmon, B.T., et al. Akt 1 polymorphisms are associated with risk for metabolic syndrome. (2011) *Hum Genet* 129(2): 129-139.  
PubMed | Crossref | Others
88. Small, E.M., O'Rourke, J.R., Moresi, V., et al. Regulation of PI3-kinase / Akt signaling by muscle-enriched microRNA-486. (2010) *Proc Natl Acad Sci U S A* 107(9): 4218-4223.  
PubMed | Crossref | Others
89. Meng, Z., Lou, S., Tan, J. et al. Nuclear factor-kappa B inhibition can enhance therapeutic efficacy of 131I on the in vivo management of differentiated thyroid cancer. (2012) *Life sci* 91(23-24): 1236-1241.  
PubMed | Crossref | Others
90. Chan, T.O., Pascal, J.M., Armen, R.S. et al. Autoregulation of kinase dephosphorylation by ATP binding in AGC protein kinases. (2012) *Cell Cycle* 11(3): 475-478.  
PubMed | Crossref | Others
91. Dey-Guha, I., Wolfer, A., Yeh, A.C., et al. Asymmetric cancer cell division regulated by Akt. (2011) *PNAS* 108(31): 12845-12850.  
PubMed | Crossref | Others
92. Santi, S.A., Lee, H. Ablation of Akt 2 induces autophagy through cell cycle arrest, the down regulation of p70S6K, and the deregulation of mitochondria in MDA-MB231 cells. (2011) *PLoS One* 6(1): e14614.  
PubMed | Crossref | Others
93. Liu, Y., Yang, G., Bu, X., et al. Cell-type-specific regulation of raft-associated Akt signaling. (2011) *Cell Death Dis* 2: e145.  
PubMed | Crossref | Others
94. Sheng, S., Qiao, M., Pardee, A.B. Metastasis and AKT activation. (2009) *J cell physiol* 218(3): 451-454.  
PubMed | Crossref | Others
95. Los, M., Maddika, S., Erb, B., et al. Switching Akt: from survival signaling to deadly response. (2009) *BioEssays* 31(5): 492-495.  
PubMed | Crossref | Others
96. Bluff, J.E., Brown, N.J., Reed, M.W., et al. Tissue factor, angiogenesis and tumor progression. (2008) *Br Cancer Res* 10(2): 204.  
PubMed | Crossref | Others
97. Schultze, S.M., Jensen, J., Hemmings, B.A., et al. Promiscuous affairs of PKB / AKT isoforms in metabolism. (2011) *Arch Physiol Biochem* 117(2): 70-77.  
PubMed | Crossref | Others
98. Shiojima, I. Role of Akt Signaling in Vascular Homeostasis and Angiogenesis. (2002) *Circ Res* 90(12): 1243-1250.  
PubMed | Crossref | Others
99. Ellsworth, R.E., Decewicz, D.J., Shriver, C.D., et al. Breast cancer in the personal genomics era. (2010) *Cur Genomics* 11(3): 146-161.  
PubMed | Crossref | Others
100. Gao, X., Lowry, P.R., Zhou, X., et al. PI3K / Akt signaling requires spatial compartmentalization in plasma membrane microdomains. (2011) *Proc Natl Acad Sci U S A* 108(35): 14509-14514.  
PubMed | Crossref | Others
101. Wullschleger, S., Sakamoto, K., Johnstone, L., et al. How moderate changes in Akt T-loop phosphorylation impact on tumorigenesis and insulin resistance. (2011) *Dis Model Mech* 4(1): 95-103.  
PubMed | Crossref | Others
102. Hanker, A.B., Der, C.J. The roles of Ras family small GTPases in Breast Cancer. (2010) *Cell Signal Coll* 2763-2772.  
PubMed | Crossref | Others
103. Omerovic, J., Laude, A.J., Prior, I.A. Ras proteins: paradigms for compartmentalized and isoform-specific signalling. (2007) *Cellular Mol Life Sci* 64(19-20): 2575-2589.

**Short title:**

Dynamics of Akt and RCAS 1 in Breast Cancer Tissues

PubMed | Crossref | Others

104. Polyak, K., Weinberg, R.A. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. (2009) *Nat Rev Cancer* 9(4): 265-273.

PubMed | Crossref | Others

105. Memmott, R.M., Dennis, P.A. Akt-dependent and -independent mechanisms of mTOR regulation in cancer. (2009) *Cellular Signalling* 21(5): 656-664.

PubMed | Crossref | Others

106. Zhang, Y., Liu, X., Zhang, J., et al. The expression and clinical significance of PI3K, pAkt and VEGF in colon cancer. (2012) *Oncol Lett* 4(4): 763-766.

PubMed | Crossref | Others

107. Dutsch-Wicherek, M., Lazar, A., Tomaszewska, R. The involvement of RCAS 1 in creating a suppressive tumor microenvironment in patients with salivary gland Adenocarcinoma. (2010) *Cancer Microenviron* 4(1): 13-21.

PubMed | Crossref | Others

108. Adamek, D., Radwańska, E., Gajda, M. Expression of RCAS 1 protein in microglia/macrophages accompanying brain tumors, an immunofluorescence study. (2009) *Folia Neuropathol* 47(3): 240-246.

PubMed | Crossref | Others

109. Du, K., Tschlis, P.N. Regulation of the Akt kinase by interacting proteins. (2005) *Oncogene* 24(50): 7401-7409.

PubMed | Crossref | Others

110. Srivastava, K., Srivastava, A., Mittal, B. Potential biomarkers in gallbladder cancer: present status and future directions. (2013) *Biomarkers* 18(1): 1-9.

PubMed | Crossref | Others

111. Kino, T., Chrousos, G.P. Tumor associated, Estrogen Receptor related Antigen EBAG9: linking intracellular vesicle trafficking, immune homeostasis and malignancy. (2009) *Mol Interv* 9(6): 294-298.

PubMed | Crossref | Others

112. Maddocks, O.D., Vousden, K.H. Metabolic regulation by p53. (2011) *J Mol Med* 89(3): 237-245.

PubMed | Crossref | Others

113. Dutsch-Wicherek, M., Tomaszewska, R., Lazar, A., et al. The associations between RCAS1 expression in laryngeal and pharyngeal cancer and its healthy stroma with cancer relapse. (2009) *BMC Cancer* 9: 35.

PubMed | Crossref | Others

114. Brunelle, J.K., Letai, A. Control of mitochondrial apoptosis by the Bcl 2 family. (2009) *J Cell Sci* 122(Pt 4): 437-441.

PubMed | Crossref | Others

115. Kumagai, J., Urano, T., Ogushi, T., et al. EBAG 9 is a tumor-promoting and prognostic factor for bladder cancer. (2009) *Intl J Cancer* 124(4): 799-805.

PubMed | Crossref | Others

116. Adams, J.R., Schachter, N.F., Liu, J.C., et al. Elevated PI3K signaling drives multiple breast cancer subtypes. (2011) *Oncotarget* 2(6): 435-447.

PubMed | Crossref | Others

117. Datta, S.R., Dudek, H., Tao, X., et al. Akt phosphorylation of BAD couples survival signals to the cell intrinsic death machinery. (1997) *Cell* 91(2): 231-241.

PubMed | Crossref | Others

118. Longuespee, R., Boyon, C., Desmons, A., et al. Ovarian cancer molecular pathology. (2012) *Cancer Metastasis Rev* 31(3-4): 713-732.

PubMed | Crossref | Others

119. Georgescu, M.-M. PTEN Tumor Suppressor Network in PI3K-Akt Pathway Control. (2010) *Genes Cancer* 1(12): 1170-1177.

PubMed | Crossref | Others

120. Jones, R.G., Thompson, C.B. Tumor suppressors and cell metabolism: a recipe for cancer growth. (2009) *Genes Dev* 23(5): 537-548.

PubMed | Crossref | Others

121. Djioque, S., Kamdje, A.H.N., Vecchio, L. Insulin resistance and cancer: the role of insulin and IGFs. (2013) *Endocr Relat Cancer* 20(1):

R1-R17.

PubMed | Crossref | Others

122. Darrington, R.S., Campa, V.M., Walker, M.M., et al. Distinct expression and activity of GSK 3 $\alpha$  and GSK 3 $\beta$  in prostate cancer. (2012) *Int J Cancer* 131(6): E872-883.

PubMed | Crossref | Others

123. Matheny, R.W. Jr., Adamo, M.L. Current perspectives on Akt activation and Akt-ions. (2009) *Exp Biol Med* 234(11): 1264-1270.

PubMed | Crossref | Others

124. Azab, S.S. Targeting the mTOR signalling pathways in breast cancer: more than the rapalogs. (2013) *J Biochem Pharmacological Res* 1(2): 75-83.

PubMed | Crossref | Others

125. Cidado, J., Park, B.H. Targeting the PI3K / Akt / mTOR pathway for breast cancer therapy. (2012) *J Mammary Gland Biol Neoplasia* 17(3-4): 205-216.

PubMed | Crossref | Others

126. Moritz, A., Li, Y., Guo, A. Akt – RSK - S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. (2010) *Sci Signal* 3(136): ra64.

PubMed | Crossref | Others

127. Blanco-Aparicio, C., Canamero, M., Cecilia, Y., et al. Exploring the gain of function contribution of Akt to mammary tumorigenesis in mouse models. (2010) *PLoS One* 5(2): e9305.

PubMed | Crossref | Others

128. Dummmler, B., Tschopp, O., Hynx, D. Life with a single isoform of Akt: mice lacking Akt 2 and Akt 3 are viable but display impaired glucose homeostasis and growth deficiencies. (2006) *Mol Cell Biol* 26(21): 8042-8051.

PubMed | Crossref | Others

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