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OPINION PAPER

Opposing roles of the oncogene Akt isoforms in tumour progression: is there a dark side to Akt pathway inhibition?

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Since the discovery of the serine/threonine kinase Akt/PKB two decades ago, it has been implicated in an increasing number of physiological and pathological processes [24]. Many studies have shown that Akt hyperactivation is a common characteristic in a wide range of human tumours [2]. Akt regulates diverse cellular processes such as apoptosis, cell proliferation, differentiation, migration and angiogenesis. Furthermore, a number of studies have shown that overexpression and/or activation of Akt renders tumour cells resistant to chemotherapeutic drugs and signalling pathway inhibitors such as Gleevec, Iressa and Herceptin [21]. siRNA-mediated knockdown of Akt significantly reduces tumor growth and invasiveness and induces apoptosis [23]. These observations have made it an attractive target for the development of anticancer therapeutics, and it has been postulated that inhibition of Akt alone or in combination with standard cancer chemotherapeutics will reduce the apoptotic threshold [12]. To date, three Akt family members have been identified in mammals. These are transcribed from distinct genetic loci, termed Akt1/PKBα, Akt2/PKBβ and Akt3/PKBγ. Akt family members share a similar domain structure [26] and all three proteins are expressed in all cells and tissues.

Oncogenic mutations in the phosphoinositide (PI) 3-kinase (PI3-K) pathway lead to hyperactivation of all three Akt isoforms [27]. Akt can be activated by steroid hormones or growth factors, downstream of constitutively active Ras and Src pathways [18]. Properly regulated activation of Akt depends on the integrity of the pleckstrin homology (PH) domain, which mediates its membrane translocation and subsequent phosphorylation at two regulatory sites, Thr308 in the kinase activation loop, and Ser473 in the C-terminal domain of the Akt family, only *AKT2* mRNA is frequently overexpressed in human cancers [17]. Activation of Akt can also result from a dominant mutation that was identified in human tumours [3] and a point mutation of *AKT2* has been reported in familial diabetes [9]. Ectopic expression of constitutively active Akt and even wild-type Akt2 results in oncogenic transformation in vitro and in vivo [4]. For these reasons, many clinical trials are underway using recently developed small molecule inhibitors targeting the PI3K/Akt pathway.

To date, more than 200 Akt substrates have been identified. However, isoform specificity has been studied for only a few, including the Akt1-specific targets p21 [13] and SKP2 [8], and the Akt2-specific targets MDM2 and AS160 [22]. Moreover, none of these targets can account for the differential effects of Akt isoforms on invasive migration [10]. A recent report raises concerns that Akt1 and Akt2 isoforms have opposing functions in the regulation of carcinoma migration. Bae et al. reported the first evidence for the different functions of Akt isoforms, from Akt1, Akt2 and Akt3 knockout mouse studies. Akt1 null mice displayed growth retardation, Akt2 null mice developed insulin-resistant diabetes (because of its dominant role in metabolic signalling in the liver), and Akt3 null mice revealed a reduced brain size. Other in vivo studies using isoform-specific RNAi or inhibitors have highlighted the different functions of the Akt isoforms, especially in modulating motility in breast cancer [1, 6, 7].

In contrast, several in vitro studies of ectopic expression of Akt proteins indicated that various Akt isoforms could stimulate motility [25] and enhance migration [11]. Consistent with the above study, other in vivo studies using Akt1 knockout mice resulted in fewer metastases, with the conclusion that Akt1 signalling is positively associated with invasion leading

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to metastasis [16]. A study of constitutively active Akt1 in the mouse mammary gland by Hutchinson *et al*. reported that Akt1 suppresses mammary tumor invasion [14]. Another in vitro assay also demonstrated that Akt1 suppresses breast cancer cell migration by enhancing the proteasomal degradation of the nuclear factor of activated T cells transcription factor, which in turn promotes the expression of invasion genes such as *COX2* [28]. More recent studies have shown that Akt1 can block cell migration through tuberous sclerosis complex 2 [19], its silencing induces epithelial-mesenchymal transition in MCF10A cells and in the same cells Akt2 actually enhances this phenotype [15]. The specific substrates of Akt2 responsible for enhancing cell migration have not yet been identified. More recently, Chin et al. reported more mechanistic insights as to how Akt isoforms differentially control cell migration in breast cancer cells, showing that the actin bundling protein palladin is an exclusive Akt1 substrate that is not phosphorylated by Akt2 and is required for efficient breast cancer cell migration [5]. A similar in vivo study from Maroulakou et al. also showed a suppression of metastasis, consistent with Akt1 functioning as a metastasis suppressor [20]. This same study also noted that knockout of Akt2 in mice decreased metastases, consistent with Akt2 functioning as an enhancer of metastasis. Moreover, it has been shown that the membrane recruitment of Akt2 in insulin-stimulated adipocytes was faster than Akt1, and was dependent on the PH domain and the Akt2 linker region [10]. Furthermore, Chin et al. showed that the Akt1 linker region determines the selectivity of Akt1 over Akt2 in the phosphorylation of palladin [5]. Whether the linker region contains specific microdomains or any other determinants that dictate Akt isoform substrate selectivity is not known. Regardless, specific substrates of Akt isoforms that are responsible for transducing distinct phenotypes clearly do exist. These new data have cast Akt itself as the lead role in the already crowded stage of Akt signalling.

In summary, there is now overwhelming evidence that Akt1 and Akt2 have opposing functions in modulating phenotypes associated with migration and invasion. Even-though the inhibition of Akt signalling should foil local tumor growth, it could promote invasion and metastasis in certain settings. Emerging evidence that the three Akt isoforms have distinct substrates with distinct physiological outcomes will require us to re-evaluate the aim of global inhibition of Akt in cancer therapy.

References

1. Bae SS, Cho H, Mu J, Birnbaum MJ (2003) Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J Biol Chem 278(49):49530–49536. doi:10.1074/jbc.M306782200
2. Bellacosa A, Testa JR, Staal SP, Tsichlis PN (1991) A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science 254(5029):274–277
3. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448(7152):439–444. doi:10.1038/nature05933
4. Cheng JQ, Altomare DA, Klein MA, Lee WC, Kruh GD, Lissy NA, Testa JR (1997) Transforming activity and mitosis-related expression of the AKT2 oncogene: evidence suggesting a link between cell cycle regulation and oncogenesis. Oncogene 14 (23):2793–2801. doi:10.1038/sj.onc.1201121
5. Chin YR, Toker A (2010) The actin-bundling protein palladin is an Akt1-specific substrate that regulates breast cancer cell migration. Mol Cell 38(3):333–344. doi:10.1016/j.molcel.2010.02.031
6. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292 (5522):1728–1731. doi:10.1126/science.292.5522.1728
7. Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ (2005) Role for Akt3/protein kinase Bgamma in attainment of normal brain size. Mol Cell Biol 25(5):1869–1878. doi:10.1128/MCB.25.5.1869-1878.2005
8. Gao D, Inuzuka H, Tseng A, Chin RY, Toker A, Wei W (2009) Phosphorylation by Akt1 promotes cytoplasmic localization of Skp2 and impairs APCCdh1-mediated Skp2 destruction. Nat Cell Biol 11(4):397–408. doi:10.1038/ncb1847
9. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I (2004) A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science 304(5675):1325–1328. doi:10.1126/science.1096706
10. Gonzalez E, McGraw TE (2009) Insulin-modulated Akt subcellular localization determines Akt isoform-specific signaling. Proc Natl Acad Sci USA 106(17):7004–7009. doi:10.1073/pnas.0901933106
11. Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, Donowitz M, Tsichlis PN, Larue L (2003) The protein

kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res 63(9):2172–2178
12. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB (2005) Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Disc 4(12):988–1004. doi:10.1038/nrd1902
13. Heron-Milhavet L, Franckhauser C, Rana V, Berthenet C, Fisher D, Hemmings BA, Fernandez A, Lamb NJ (2006) Only Akt1 is required for proliferation, while Akt2 promotes cell cycle exit through p21 binding. Mol Cell Biol 26(22):8267–8280. doi:10.1128/MCB.00201-06
14. Hutchinson JN, Jin J, Cardiff RD, Woodgett JR, Muller WJ (2004) Activation of Akt-1 (PKB-alpha) can accelerate ErbB-2-mediated mammary tumorigenesis but suppresses tumor invasion. Cancer Res 64(9):3171–3178
15. Irie HY, Pearline RV, Grueneberg D, Hsia M, Ravichandran P, Kothari N, Natesan S, Brugge JS (2005) Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. J Cell Biol 171(6):1023–1034. doi:10.1083/jcb.200505087
16. Ju X, Katiyar S, Wang C, Liu M, Jiao X, Li S, Zhou J, Turner J, Lisanti MP, Russell RG, Mueller SC, Ojeifo J, Chen WS, Hay N, Pestell RG (2007) Akt1 governs breast cancer progression in vivo. Proc Natl Acad Sci USA 104(18):7438–7443. doi:10.1073/pnas.0605874104
17. Kim D, Dan HC, Park S, Yang L, Liu Q, Kaneko S, Ning J, He L, Yang H, Sun M, Nicosia SV, Cheng JQ (2005) AKT/PKB signaling mechanisms in cancer and chemoresistance. Front Biosci 10:975–987
18. Liu AX, Testa JR, Hamilton TC, Jove R, Nicosia SV, Cheng JQ (1998) AKT2, a member of the protein kinase B family, is activated by growth factors, v-Ha-ras, and v-src through phosphatidylinositol 3-kinase in human ovarian epithelial cancer cells. Cancer Res 58(14):2973–2977
19. Liu H, Radisky DC, Nelson CM, Zhang H, Fata JE, Roth RA, Bissell MJ (2006) Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. Proc Natl Acad Sci USA 103(11):4134–4139. doi:10.1073/pnas.0511342103
20. Maroulakou IG, Oemler W, Naber SP, Tsichlis PN (2007) Akt1 ablation inhibits, whereas Akt2 ablation accelerates, the development of mammary adenocarcinomas in mouse mammary tumor virus (MMTV)-ErbB2/neu and MMTV-polyoma middle T transgenic mice. Cancer Res 67(1):167–177. doi:10.1158/0008-5472.CAN-06-3782
21. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6(2):117–127. doi:10.1016/j.ccr.2004.06.022
22. Ng Y, Ramm G, Lopez JA, James DE (2008) Rapid activation of Akt2 is sufficient to stimulate GLUT4 translocation in 3T3-L1 adipocytes. Cell Metab 7(4):348–356. doi:10.1016/j.cmet.2008.02.008
23. Remy I, Montmarquette A, Michnick SW (2004) PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. Nat Cell Biol 6(4):358–365. doi:10.1038/ncb1113
24. Staal SP (1987) Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA 84(14):5034–5037
25. Tanno S, Mitsuuchi Y, Altomare DA, Xiao GH, Testa JR (2001) AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. Cancer Res 61(2):589–593
26. Testa JR, Bellacosa A (2001) AKT plays a central role in tumorigenesis. Proc Natl Acad Sci USA 98(20):10983–10985. doi:10.1073/pnas.211430998
27. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol 11(5):329–341. doi:10.1038/nrm2882
28. Yoeli-Lerner M, Chin YR, Hansen CK, Toker A (2009) Akt/protein kinase b and glycogen synthase kinase-3beta signaling pathway regulates cell migration through the NFAT1 transcription factor. Mol Cancer Res 7(3):425–432. doi:10.1158/1541-7786.MCR-08-0342