Hepatic Oncology

Isoform-specific activation of Akt involvement in hepatocarcinogenesis

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Akt signaling in carcinogenesis: isoform specificity

Akt has emerged as a critical signaling node within all eukaryotic cells. Its deregulation may result in severe disorders. Phosphorylation of several amino acids within different AKT protein domains is responsible for its activation. This is triggered by different stimuli, either through a PI3K (class Ia) dependent pathway triggered by tyrosine kinase receptors or through mTORC2. mTORC2 is an intracellular complex promoting cell proliferation and survival through phosphorylation of AGC kinase family members, including Akt [1]. Cellular phosphatases, for example, PHLPP, are also involved in Akt regulation.

Mammalian cells contain three genes that encode three closely related and highly conserved isoforms of Akt, including PKB α /Akt-1, PKB β /Akt-2 and PKB γ /Akt-3. They all share a highly conserved N-terminal pleckstrin homology (PH) domain, a serine-threonine kinase catalytic domain and a small carboxyterminal

regulatory domain. Akt1 is ubiquitously expressed, whereas Akt2 is predominantly expressed in insulin-sensitive organs. Akt3 expression is restricted to the brain and testis.

Because of their high sequence homology, Akt isoforms were thought to be redundant in their biological functions. In fact, recent literature showed that Akt functions are isoform-specific and play different, sometimes opposite, roles in metabolism and growth [2,3]. Mouse mutant models lacking individual Akt isoforms were constructed to decipher their specific functions. Mice lacking Akt-1 exhibited abnormal growth with a reduction in body-weight [4,5]. In contrast, Akt-2-deficient mice had normal growth but a diabetes-like syndrome with an increase of fasting plasma glucose level, elevated hepatic glucose output and peripheral insulin resistance [4,6]. Akt-3-deficient mice exhibited a reduction in brain weight but maintained a normal glucose homeostasis and body-weight [7]. Altogether, these data suggest different biological roles for the various

KEYWORDS

- AKT • cancer • HCC • HCV
- isoform • liver

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Akt isoforms. However, double knock-out mice revealed more complex overlapping functions.

The cascade of downstream events resulting of Akt isoform activation might be dependent upon the Akt stimulus and substrate specificity, tissue distribution and subcellular localization and regulatory partners. In the past 10 years, several studies supported the hypothesis that Akt isoforms play specific roles in cancer initiation, growth and metastasis. For example, in mouse myogenic cells, Akt1 regulated G1/S transition, while Akt2 did not [8], witnessing the different effects of Akt isoforms in cell cycle progression. Akt1 induced mammary tumor initiation and growth, while Akt2 exerted the opposite effect [9]. This suggests that Akt1 plays an essential role in breast cancer induction while Akt2 is primarily involved in metastatic dissemination [10]. In the case of colorectal cancer, metastasis was inhibited by Akt2 expression knock-down, but it was not rescued by Akt1 overexpression [11]. In mouse embryonic fibroblasts, podosome formation induced by Src (which is linked to the metastatic process) was promoted by Akt1, whereas Akt3 suppressed it. Akt2 did not appear to play a significant role in this process. Interestingly, Akt1 and Akt3 both suppressed, whereas Akt2 enhanced, phorbol-ester-induced podosome formation [12]. In the context of lung carcinogenesis, Akt1 gene knockout delayed the initiation and tumor growth, whereas Akt2 gene knockout accelerated tumorigenesis. Deletion of the Akt3 gene had no significant effect on tumor induction and growth [13]. This suggests minimal functional overlap between Akt isoforms in the context of lung tumor initiation. In the androgen-sensitive cell line LNCaP, the ablation of Akt1 or Akt2 induced apoptosis; in androgen-independent LNCaP cells, Akt1 gene deletion had less effects and Akt2 ablation had none [14]. Phung and collaborators showed that Akt1 promoted and Akt3 inhibited vascular tumor growth [15]. Finally, in colon, bladder and laryngeal carcinoma derived cells, inhibition of Akt1 using siRNA reduced survival after tumor cell radiation, whereas inhibition of Akt2 or 3 was less effective. [16]. The above studies suggest that selective inhibition of Akt isoforms could be important in anticancer drug development.

Published data suggest a trend toward a cancer promoting effect of Akt1, while the other isoforms display opposite or insignificant effects. This suggests that the Akt kinase activity specificity is driven by its polymorphism. Akt isoform

substrate specificity was first investigated in the case of glucose intracellular transport regulation through Glut4 translocation mechanisms. Katome *et al.* showed that Akt2, not Akt1, was the principal isoform responsible for the stimulation of Glut4 translocation in adipocytes [17]. Interestingly, they observed that the subcellular localization of Akt2 to the Glut4 vesicles was essential. Akt1 aberrant localization to plasma membrane, through E17K mutation, was associated with Akt2-like signaling regarding Glut4 translocation [3]. Akt2 specifically phosphorylates AS160, which was involved in Rab-dependent Glut4 translocation mechanisms in adipocytes and muscle cells. More recently, palladin (actin-associated protein) and prohibitin-1 have been described as Akt1-specific substrates in breast cancer cells [18] and pancreatic cells [19], respectively. In muscle cells, p21 was specifically phosphorylated by Akt1, while Akt-2 only bound to p21 [8]. Pixt2 was defined as a specific substrate for Akt2, involved in the switch from proliferation to differentiation in C2C12 muscle cells [20]. In fact, data demonstrating Akt isoform substrate specificity are scarce. Another explanation for specific Akt isoform functions may lie in their subcellular localization, in other words, local availability. However, data on Akt subcellular localization are still controversial. Akt1 was often observed associated to membranes in the cytoplasm. Depending on the studies, Akt2 was found within the nucleus or in mitochondria. Upon insulin-receptor activation, Akt2 accumulated transiently at the plasma membrane. Interestingly, ablation of single Akt isoforms did not alter the subcellular localization of the remaining isoforms [21]. Furthermore, the swap of Akt1 to Akt2 PH domain did not confer enhanced insulin-induced Akt1 plasma membrane localization. Thus, more studies are needed to explore the subcellular availability of Akt and a potential link to isoform-specific functions.

Activation of Akt isoforms in liver cancer

Hepatocellular carcinoma (HCC) is the most common primary liver carcinoma, often occurring in patients with chronic liver injury. The main risk factors for HCC development include chronic viral hepatitis (HBV and HCV), chronic alcoholic liver disease; nonalcoholic steatohepatitis, autoimmune hepatitis, genetic metabolic diseases and exposure to carcinogens. During the last decade, the molecular mechanisms of

liver carcinogenesis have been intensively investigated. Gene expression and signaling pathway alterations have been studied using HCC biopsies or resections and high-throughput technologies to identify HCC molecular signatures [22]. These studies suggested different potential molecular mechanisms for liver carcinogenesis according to the etiology, involving somatic mutations in p53, Wnt/ β -catenin and telomerase-related genes [22,23]. Despite the very high frequency of Akt activation in tumoral tissues (e.g., 71% of HCC, including HCV-induced HCC [24]), Pi3-K/Akt somatic mutations were rarely found in these studies [22]. Thus, the molecular mechanisms responsible for aberrant Akt activity in HCC tissues result of complex deregulations of pathways that control Akt activation.

Recent data demonstrated that overexpression of a myristoylated Akt-1 (which is constitutively active) in mouse livers, induced lipogenesis and cell proliferation, leading to hepatocellular carcinoma development through mTORC1 dependent and independent pathways [25]. Hyperactivation of mTOR signaling was found in approximately 50–60% in HCC [26]. Grabinski and collaborators inhibited mTOR in different human hepatoma cell lines and observed differential isoform-specific activation of Akt1, 2 and 3. Knockdown of Akt1 led to a significant inhibition of proliferation of all tested HCC cell lines. Furthermore, while depletion of Akt2 diminished the proliferation of both Hep3B and HepG2 cells, knockdown of Akt3 had no significant effect on Hep3B cells proliferation. In human HCC tissue samples,

Akt isoforms were differentially activated, but all Akt isoforms were activated in an HCC patient with mutated PI3KR1 or CA [27]. Another study showed that the Akt2 isoform was upregulated in HCC and a significant prognostic marker [28]. In our hands, an *in vivo* transgenic mouse model for HCV carcinogenesis displayed activation of Akt1 kinase activity in livers expressing the entire repertoire of HCV proteins, while Akt2 activity was unperturbed in these livers [IMACHE MR ET AL., UNPUBLISHED DATA].

Together, these data suggest that different Akt isoforms play different roles in hepatocarcinogenesis. This is in keeping with the scenarios described in other human carcinomas, but remains to be fully demonstrated. The opposing roles of Akt1 and Akt2 in carcinogenesis, while both are activated by PI3K, may explain the poor efficacy of PI3K inhibitors in the treatment of HCC. Regarding the paucity of current data on differential Akt isoform activation in HCC, more studies will be needed to unravel the molecular mechanisms involved and to define potential therapeutic targets.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the RICTOR–mTOR complex. *Science* 307(5712), 1098–1101 (2005).
- Heron-Milhavet L, Khouya N, Fernandez A, Lamb NJ. Akt1 and Akt2: differentiating the aktion. *Histol. Histopathol.* 26(5), 651–662 (2011).
- Gonzalez E, McGraw TE. The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle* 8(16), 2502–2508 (2009).
- Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKB α is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J. Biol. Chem.* 276(42), 38349–38352 (2001).
- Chen WS, Xu PZ, Gottlob K *et al.* Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes Dev.* 15(17), 2203–2208 (2001).
- Garofalo RS, Orena SJ, Rafidi K *et al.* Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB β . *J. Clin. Invest.* 112(2), 197–208 (2003).
- Easton RM, Cho H, Roovers K *et al.* Role for Akt3/protein kinase B γ in attainment of normal brain size. *Mol. Cell. Biol.* 25(5), 1869–1878 (2005).
- Heron-Milhavet L, Franckhauser C, Rana V *et al.* Only Akt1 is required for proliferation, while Akt2 promotes cell cycle exit through p21 binding. *Mol. Cell. Biol.* 26(22), 8267–8280 (2006).
- Maroulakou IG, Oemler W, Naber SP, Tschlis PN. 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 (2007).
- Dillon RL, Marcotte R, Hennessy BT, Woodgett JR, Mills GB, Muller WJ. Akt1 and akt2 play distinct roles in the initiation and metastatic phases of mammary tumor progression. *Cancer Res.* 69(12), 5057–5064 (2009).
- Rychahou PG, Kang J, Gulhati P *et al.* Akt2 overexpression plays a critical role in the establishment of colorectal cancer metastasis. *Proc. Natl Acad. Sci. USA* 105(51), 20315–20320 (2008).

- 12 Eves R, Oldham R, Jia L, Mak AS. The roles of akt isoforms in the regulation of podosome formation in fibroblasts and extracellular matrix invasion. *Cancer* 7(1), 96–111 (2015).
- 13 Linnerth-Petrik NM, Santry LA, Petrik JJ, Wootton SK. Opposing functions of Akt isoforms in lung tumor initiation and progression. *PLoS ONE* 9(4), e94595 (2014).
- 14 Cariaga-Martinez AE, Lopez-Ruiz P, Nombela-Blanco MP *et al.* Distinct and specific roles of AKT1 and AKT2 in androgen-sensitive and androgen-independent prostate cancer cells. *Cell. Signal.* 25(7), 1586–1597 (2013).
- 15 Phung TL, Du W, Xue Q *et al.* Akt1 and akt3 exert opposing roles in the regulation of vascular tumor growth. *Cancer Res.* 75(1), 40–50 (2015).
- 16 Kim IA, Bae SS, Fernandes A *et al.* Selective inhibition of Ras, phosphoinositide 3 kinase, and Akt isoforms increases the radiosensitivity of human carcinoma cell lines. *Cancer Res.* 65(17), 7902–7910 (2005).
- 17 Katome T, Obata T, Matsushima R *et al.* Use of RNA interference-mediated gene silencing and adenoviral overexpression to elucidate the roles of AKT/protein kinase B isoforms in insulin actions. *J. Biol. Chem.* 278(30), 28312–28323 (2003).
- 18 Chin YR, Tokar A. The actin-bundling protein palladin is an Akt1-specific substrate that regulates breast cancer cell migration. *Mol. Cell* 38(3), 333–344 (2010).
- 19 Han EK, Mcgonigal T, Butler C, Giranda VL, Luo Y. Characterization of Akt overexpression in MiaPaCa-2 cells: prohibitin is an Akt substrate both *in vitro* and in cells. *Anticancer Res.* 28(2A), 957–963 (2008).
- 20 Gherzi R, Trabucchi M, Ponassi M, Gallouzi IE, Rosenfeld MG, Briata P. Akt2-mediated phosphorylation of Pitx2 controls Ccnd1 mRNA decay during muscle cell differentiation. *Cell Death Different.* 17(6), 975–983 (2010).
- 21 Santi SA, Lee H. The Akt isoforms are present at distinct subcellular locations. *Am. J. Physiol. Cell. Physiol.* 298(3), C580–C591 (2010).
- 22 Boyault S, Rickman DS, De Reynies A *et al.* Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 45(1), 42–52 (2007).
- 23 Nault JC, Mallet M, Pilati C *et al.* High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat. Commun.* 4, 2218 (2013).
- 24 Higgs MR, Lerat H, Pawlowsky JM. Hepatitis C virus-induced activation of beta-catenin promotes c-Myc expression and a cascade of pro-carcinogenetic events. *Oncogene* 32(39), 4683–4693 (2012).
- 25 Ho C, Wang C, Mattu S *et al.* AKT (v-akt murine thymoma viral oncogene homolog 1) and N-Ras (neuroblastoma ras viral oncogene homolog) coactivation in the mouse liver promotes rapid carcinogenesis by way of mTOR (mammalian target of rapamycin complex 1), FOXM1 (forkhead box M1)/SKP2, and c-Myc pathways. *Hepatology* 55(3), 833–845 (2012).
- 26 Villanueva A, Chiang DY, Newell P *et al.* Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 135(6), 1972–1983, 1983.e1971–1911 (2008).
- 27 Grabinski N, Ewald F, Hofmann BT *et al.* Combined targeting of Akt and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. *Mol. Cancer* 11, 85 (2012).
- 28 Xu X, Sakon M, Nagano H *et al.* Akt2 expression correlates with prognosis of human hepatocellular carcinoma. *Oncol. Rep.* 11(1), 25–32 (2004).