Editorial

Akt1/Akt2 inhibition and development of hepatocellular carcinoma

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The serine/threonine kinase Akt is a key regulator of important normal cellular activities, including survival, proliferation, differentiation, cell size and glucose metabolism. Akt has also a relevant role in the epithelial–mesenchymal transition during development and tumorigenesis and is a pivotal controller of angiogenesis (1,2). When deregulated, Akt can contribute to cancer development, invasion/metastasis and drug resistance acquisition. It is frequently hyper-activated in human cancer and therefore it is considered an attractive target for therapies based on small molecule inhibitors (3).

Diverse mechanisms activate Akt and many components of its pathway are now known to act either as oncoproteins or tumor suppressors. Akt is recognized as a central component in the signaling pathway composed of upstream phosphatidylinositol 3-kinase (PI3K) and PTEN (phosphatase and tensin homolog deleted on chromosome 10), and downstream tuberous sclerosis complex 2 (TSC2), eIF4E, and forkhead box O (FOXO) family of transcription factors, which represents one of the major AKT substrates (4). Several of these proteins (Akt, PI3K p110 α catalytic subunit, and eIF4E) exert mostly oncogenic activity when over-expressed or mutated, while others (PTEN, FOXO, LKB1, TSC1/TSC2, NF1, and VHL) behave as tumor suppressors.

Another downstream substrate, the mammalian target of rapamycin (mTOR) kinase, signals to the translational machinery. Activation of protein synthesis by mTOR is important for regulating cell size, proliferation and survival. Ruggero and Sonenberg (5) reported emerging molecular and genetic evidence that deregulation of specific or global protein synthesis may be a crucial mechanism leading to tumorigenesis. The tumor suppressors TSC2 and TSC1 physically interact to inhibit mTOR signaling, and phosphorylation of TSC2 by Akt results in disruption of the TSC1/TSC2 complex and activation of mTOR. Akt has also a relevant role in the epithelial–mesenchymal transition during development and cancer; in particular it is involved in the regulation of E-cadherin expression and in cell motility and invasiveness (6).

There is also evidence that Akt signaling pathway is important in tumor development, disease aggressiveness and drug resistance. In recent years increasing attention has been paid to molecular targeting the components of this pathway. Several molecules designed to specifically target PI3K-Akt have been developed: they induce cell cycle arrest or apoptosis in human cancer cells in vitro and *in vivo*. Currently there are multiple clinical trials aimed at assessing the efficacy of PI3K/Akt inhibitors in cancer patients (7,8).

Akt recognizes three isoforms encoded by separate genes. They share greater than 80% identity at the amino acid level; their relative expression differs in various mammalian tissues. Studies on isoform specific knockout mice revealed their distinct functional roles involving diverse signaling cascades, thereby controlling cell growth, metabolism, cell proliferation and survival (9,10). What is not well understood is their contribution to tumorigenesis and most of all, the different effect of their deficiencies on cancer development. Pan-PI3K and pan-Akt inhibitors that interfere with the activity of the three Akt isoforms (Akt1, Akt2 and Akt3) have recently developed (11,12). As their use has been associated, especially in dose-escalating studies, with relevant side effects it is mandatory to understand all the possible consequences of their action, before they can enter clinical use. Genetic studies in mice have demonstrated that the Akt1 ablation can substantially impair carcinogenesis (13-15) while Akt2 deletion does not inhibit cancer development (15). The combined effect of systemic deletions of Akt isoforms in adult mice has

not been explored so far as the majority of earlier studies utilized germ line deletion of Akt isoforms. However, germ line deletion of both Akt1 and Akt2 is neonatal lethal, and the deletion of both Akt1 and Akt3 is embryonic lethal, while the deletion of both Akt2 and Akt3 does not cause lethality (16). Therefore, in order to have an experimental model that can recreate the events in tumor progression and the possible effects of drug therapy *in vivo*, Wang *et al.* (17) in this issue of Cancer Cell have described a complex adult mouse model which explores the physiological consequences of different combination of systemic deletions of Akt isoforms. Specific knockout mice were used: $Akt1^{f/f}$; $Akt2^{-/-}$; $R26Cre^{ERT2}$ and $Akt1^{f/f}$; $Akt3^{-/-}$; $R26Cre^{ERT2}$ mice were generated by crossing $Akt1^{f/f}$; $R26Cre^{ERT2}$ with either $Akt2^{-/-}$ or $Akt3^{-/-}$ mice. Cre^{ERT2} is an inducible Cre recombinase fused in frame to a mutated ligand-binding domain of estrogen receptor, which responds only to hydroxytamoxifen (4-OHT) and not to estrogen. Therefore a construct expressing Cre recombinase fused with a mutated estrogen receptor ligand-binding domain that can be activated by tamoxifen (Cre^{ERT2}) was knocked into the Rosa26 (R26) locus. Thus, upon injection of tamoxifen, Cre is activated to systemically delete the floxed allele. $Akt1^{hep-/-}$; $Akt2^{-/-}$ mice were generated by crossing $Akt1^{f/f}$; $Akt2^{-/-}$ with the albumin-CRE mouse strain.

Wang *et al.* (17) with their experiments elegantly reproduced the paradigm of liver damage followed by inflammation and hepatocellular carcinoma (HCC) development (18). Their very relevant finding is the demonstration of the rapid onset of spontaneous HCC after deletion of hepatic Akt1 and systemic Akt2 in adult mice. As mentioned above, Akt is the most frequently activated oncoprotein in human cancer, and it is implicated in the genesis of HCC (19,20). Akt1 ablation is known to impair carcinogenesis (13-15). Akt2 is the major Akt isoform expressed in the liver, and its deletion substantially reduces hepatic Akt activity and the occurrence of HCC in c-Met-transfected mice (21). It is therefore somewhat surprising that the combined deletion of hepatic Akt1 and Akt2 was followed by rapid HCC onset. As the hepatocyte-specific deletion of one Akt1 allele with complete Akt2 deletion was instead not followed by HCC development, the Authors concluded that only extremely low levels of hepatic Akt activity are followed by emergence of HCC. At present, it is not known whether maximal pharmacological Akt inhibition in humans, although reversible, could facilitate instead than inhibit carcinogenic Akt activity. Certainly, some of the intestinal and metabolic consequences of Akt1/Akt2 inhibition described in this study are similar to the adverse events noted in some patients receiving MK-2206 in dose-escalation studies (22, 23). The hypothesis put forward by the Authors to explain this unexpected result opens an interesting scenario. They observed that the deletion of both Akt1 and Akt2 was associated with a transient increase followed by a marked decrease in glucose and insulin levels and subsequent decrease in body weight and hypoglycemia, with rapid and constant death of the animals. Interestingly, they observed a dramatic loss of fat tissue after systemic deletion of both Akt1 and Akt2, suggesting the utilization of fat for fatty acid oxidation and possibly a block in adipocyte differentiation, as observed in newborn mice carrying a germline deletion of both Akt1 and Akt2 (24,25). Akt1 and Akt2 deletion determined altered glucose tolerance, elevated insulin levels, elevated levels of ALT, AST, serum IL6 (with resulting increase of STAT3 phosphorylation in hepatocytes), and increase of liver tumor necrosis factor alpha mRNA, suggesting marked inflammation, possibly as a consequence of tissue damage. The liver injury and inflammation appeared to be largely dependent on increased FoxO1 activity, which occurs when Akt function is lost. These data are consistent with the experimental models in which obesity and fatty liver promote HCC (17). In these models, HCC develops despite Akt inhibition in the liver. These data can be of relevant interest for Patients who recognize obesity, non–alcoholic steatohepatitis (NASH) and diabetes as etiologic factors for HCC. This will be extremely relevant in the near future as dysmetabolic etiology is rapidly becoming predominant over HBV or HCV infections as a risk factor for HCC.

In conclusion, the study of Wang *et al.* (17) provides interesting data on the potential side effects of the systemic inhibition of both Akt1 and Akt2. Although pharmacological inhibition by pan-Akt inhibitors is potentially reversible, what is not known is whether the changes occurring during therapy could be sufficient to start a chain of events leading to HCC despite stopping the drug. Of further concern, is the recognized occurrence of HCC in obesity despite hepatic Akt inhibition. In NASH-associated HCCs, pan-PI3K/Akt inhibitors could determine a paradoxical effect, potentially increasing, instead than decreasing, the risk of HCC recurrence. Further studies on $Akt1^{hep-/-}$; $Akt2^{-/-}$ mice made obese may help addressing this question.

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