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Review

Akt pathway as a target for therapeutic intervention in HNSCC

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Summary. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common form of cancer worldwide. One frequent alteration found in this type of cancer is overactivation of the PI3K/PTEN/mTOR pathway, of which protein kinase B (PKB)/Akt is a central key element, controlling important cellular processes such as metabolism, cell size, proliferation and apoptosis, ultimately regulating cell growth and survival. Thus, drugs that target Akt directly or elements of the pathway are plausible candidates for cancer treatment. Accordingly, numerous clinical trials in various phases are being performed for these drugs. In this review, we discuss the tumorigenic capacity of Akt and focus on its role in HNSCC, paying special attention to the current efforts in treating this cancer in a more specific, Akt-targeted way, based on its primordial role in this type of cancer.

Key words: Akt, Head and neck squamous cell carcinoma, Keratinocytes

Squamous cell carcinomas

Squamous cell carcinomas (SCCs) are tumors that arise primarily from stratified epithelial cells, such as epidermis and some areas of the respiratory and digestive tracts. SCCs can be found in localizations as diverse as skin, esophagus, larynx, cervix and oral cavity, and due to altered differentiation, or metaplasia, in lung and breast. Stratified epithelia are composed of several layers of cells, called keratinocytes, which form a highly organized structure; in a typical cornified squamous stratified epithelium, such as the epidermis of the skin, four layers can be distinguished: basal, spinous, granular and corneum. The basal layer is composed of cells with proliferative potential that give rise to all the cells in the tissue. The spinous stratum lies on the basal and is composed of several layers of polyhedric cells, tightly jointed by means of desmosomes. These cells are engaged in a finely regulated program of differentiation. In the granulous layer the cells are characterized by their cytoplasmic granules. Finally, the corneous layer is composed of dead, flattened, anucleated cells that are gradually shed. This stratum is absent in some squamous stratified epithelia (e.g., those lining the internal organs) probably due to the fact that they are not subject to mechanical stress.

Each epithelial layer can be easily characterized by the expression of specific proteins, which are also indicative and distinctive of the differentiation state. Among them, it is remarkable the different expression pattern of keratins or cytokeratins. They are members of the superfamily of intermediate filaments, and confer mechanical resilience to the epithelial cells. The pair K5-K14 is expressed in the basal, proliferative layer (Byrne et al., 1994) and the pairs K1-K10 and K4-K13 in the spinous layers of cornified and non-cornified epithelia respectively (Gimenez-Conti et al., 1990; Heyden et al., 1992). In internal stratified epithelia and in hyperproliferative situations, the keratin pair K6-K16 is suprabasally expressed, and yet others are putative stem cell markers (K15) (Liu et al., 2003; Cotsarelis, 2006). The keratin expression pattern is not neutral and seems to affect essential cell functions, including proliferation, apoptosis and signal transduction (Yamamoto et al., 1986; Paramio and Jorcano, 2002; Coulombe et al., 2004). In epidermis, other characteristic proteins are involucrin, which is expressed in the upper spinous layers, and loricrin and filaggrin, which are expressed in the granular layer.

It is generally agreed that cancer arises when cells acquire mutations in three types of genes: proto-

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oncogenes, tumor suppressor genes and DNA repair genes (Vogelstein and Kinzler, 2004). Alterations in these genes result in the cell having a growth advantage with respect to a normal cell, leading to its increased proliferation and/or decreased apoptosis. For a human cell to become cancerous, a minimum of five or six mutations must occur, which collectively lead to self-sufficiency in growth signals, insensitivity to anti-growth signals, capacity of evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis (Hanahan and Weinberg, 2000). Some cells are more prone than others to suffer these changes; this would be the case for keratinocytes, since they form part of tissues which are constantly renewing and are more exposed to environmental detrimental agents than other cell types. Thus, about 80% of all tumors are of epithelial origin, of which more than half are non melanoma skin cancers. These last comprise two main types: the more frequent and less aggressive basal cell carcinoma (BCCs), and the less frequent but more aggressive and with higher metastatic potential squamous cell carcinomas (SCCs).

During tumor progression, several tissue alterations can be histologically distinguished: hyperplasia, dysplasia, carcinoma in situ, invasive carcinoma and metastasis. In hyperplastic epithelia, cells are hyperproliferative, but they are morphologically normal and tissue structure is preserved, albeit with an increased number of cells. When the epithelium becomes dysplastic, the cells begin to show an altered morphology and the tissue structure becomes slightly disrupted. In carcinomas in situ, cells are already highly proliferative and disorganized but they are still confined to their original localization. Invasive carcinomas are those carcinomas whose cells break the basal membrane and begin to invade the subjacent tissue. Finally, metastasis occurs when tumor cells reach the blood or lymphatic vessels, travel to other organs and form secondary tumors that can be either near the original tumor (local metastasis) or in distant organs (distant metastasis). Remarkably, metastases are responsible for 90% of all cancer-related deaths (Sporn, 1996).

AKT

Akt, also known as PKB, is a serine-threonine protein kinase family formed by three members (Akt1/PKBα, Akt2/PKBß and Akt3/PKBγ) that share high sequence homology and are differentially expressed at both the messenger RNA and protein levels (Bellacosa et al., 1993; Altomare et al., 1995; Brodbeck et al., 1999). Their structure comprises an amino terminal pleckstrin-homology domain, which mediates the binding of the protein to phospholipids in the plasma membrane, a central catalytic domain, with serine and threonine specific kinase activity, and a carboxyl terminal regulatory domain rich in prolines that includes a key serine residue crucial to its activation.

Akt/PKB is central in the phosphatidylinositol 3' kinase (PI3K) signaling pathway (Fig. 1). PI3K is activated by tyrosine-kinase transmembrane receptors and other signaling intermediates, such as Ras oncogenes and G proteins (Rodriguez-Viciana et al., 1994). PI3K then phosphorylates PtdIns(4,5)P_2 (PIP2) yielding PtdIns(3,4,5)P_3 (PIP3), which serves as an anchoring for intracellular proteins (primarily mediated by pleckstrin homology domains), including Akt amongst others. Membrane-bound Akt is phosphorylated in a threonine residue in the catalytic domain by the kinase PDK1, which also binds PIP3; this results in partial activation of Akt, which becomes fully activated only after the subsequent phosphorylation of a key serine residue in the regulatory domain (Stokoe et al., 1997; Stephens et al., 1998). PIP3 are converted back into PIP2 through the action of the lipid phosphatase PTEN, thus terminating the PI3K-initiated signal and avoiding further Akt activation.

Once Akt is phosphorylated and fully activated, it is capable of phosphorylating multiple substrates, producing either their functional activation or inhibition (Fig. 2). Akt substrates participate in diverse cellular processes, such as metabolism (GSK3ß, GLUT4…), proliferation (p27, CycD1, Foxo3a…), survival (Bad, caspase 9…) and cell growth (mTOR, PDE3B). Some of the key Akt activities lead to enhanced cell proliferation

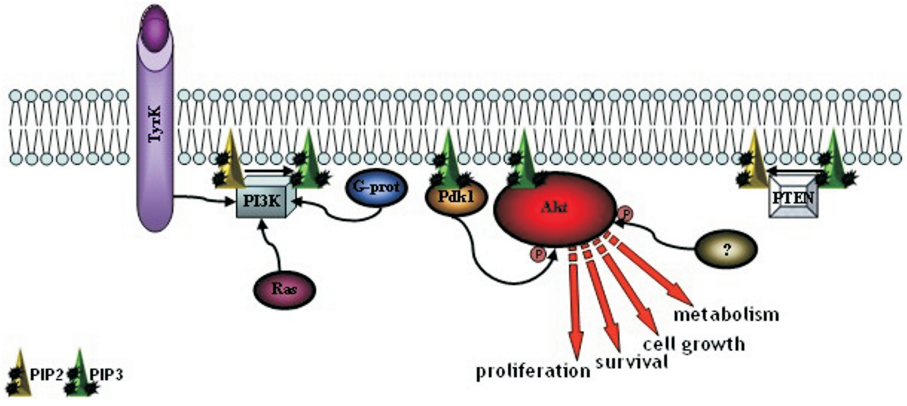

Fig. 1. Activation of Akt. PI3K is activated by ligand-bound tyorsine-kinase transmembrane receptors or alternatively by other signaling intermediates. It then phosphorylates PIP2, generating PIP3, to which both Akt and its activator PDK1 bind; PIP3 is converted back to PIP2 by the phosphatase PTEN. Phosphorylated, active Akt is then capable of phosphorylating its substrates, which participate in many cellular processes.

and decreased apoptosis, and thus Akt is considered a proto-oncogene. However, for Akt to be fully oncogenic, it must have an elevated or deregulated kinase activity (Altomare and Testa, 2005; Bellacosa et al., 2005; Song et al., 2005; Manning and Cantley, 2007).

Alterations of Akt proteins in human cancer

Since the initial isolation and identification of its viral homolog, v-Akt, as a transforming fusion protein including sequences of the viral *gag* gene (Staal, 1987; Bellacosa et al., 1991), *Akt* family members have been found to be altered in many tumor types (Table 1). For instance, Akt1 is over-activated in breast, ovary, prostate and head and neck cancer (Sun et al., 2001b; Amornphimoltham et al., 2004; Segrelles et al., 2006); Akt2 is over-activated in ER+ breast, ovary and head and neck tumors (Yuan et al., 2000; Sun et al., 2001a; Pedrero et al., 2005), over-expressed in thyroid cancer (Ringel et al., 2001) and amplified and over-expressed in ovary, head and neck and pancreas tumors (Cheng et al., 1992, 1996; Bellacosa et al., 1995; Pedrero et al., 2005); and Akt3 is over-expressed in ER- breast tumors (Nakatani et al., 1999). However, although it has been found frequently activated, no mutations in Akt genes have been reported until recently, when an activating lysine to glutamic acid substitution in the PH domain (E17K) of Akt1 was identified in some breast, colorectal and ovarian cancers (Carpten et al., 2007).

Table 1. Alterations of Akt isoforms in human tumors.

ISOFORM	TUMOR	ALTERATION	REFERENCES
Akt1	Breast	Over-activation	(Sun et al., 2001b)
	Ovary		
	Prostate		
	Head and neck	Over-activation	(Amornphimoltham et al., 2004; Segrelles et al., 2006)
Akt2	Breast (ER+)	Over-activation	(Sun et al., 2001a)
	Head and neck	Amplification Over-activation	(Pedrero et al., 2005)
	Ovary	Amplification Over-expression	(Cheng et al., 1992; Bellacosa et al., 1995)
		Over-activation	(Yuan et al., 2000)
	Thyroid	Over-expression	(Ringel et al., 2001)
	Pancreas	Amplification	(Cheng et al., 1996)
Akt3	Brast (ER-)	Amplification Over-expression	(Nakatani et al., 1999)

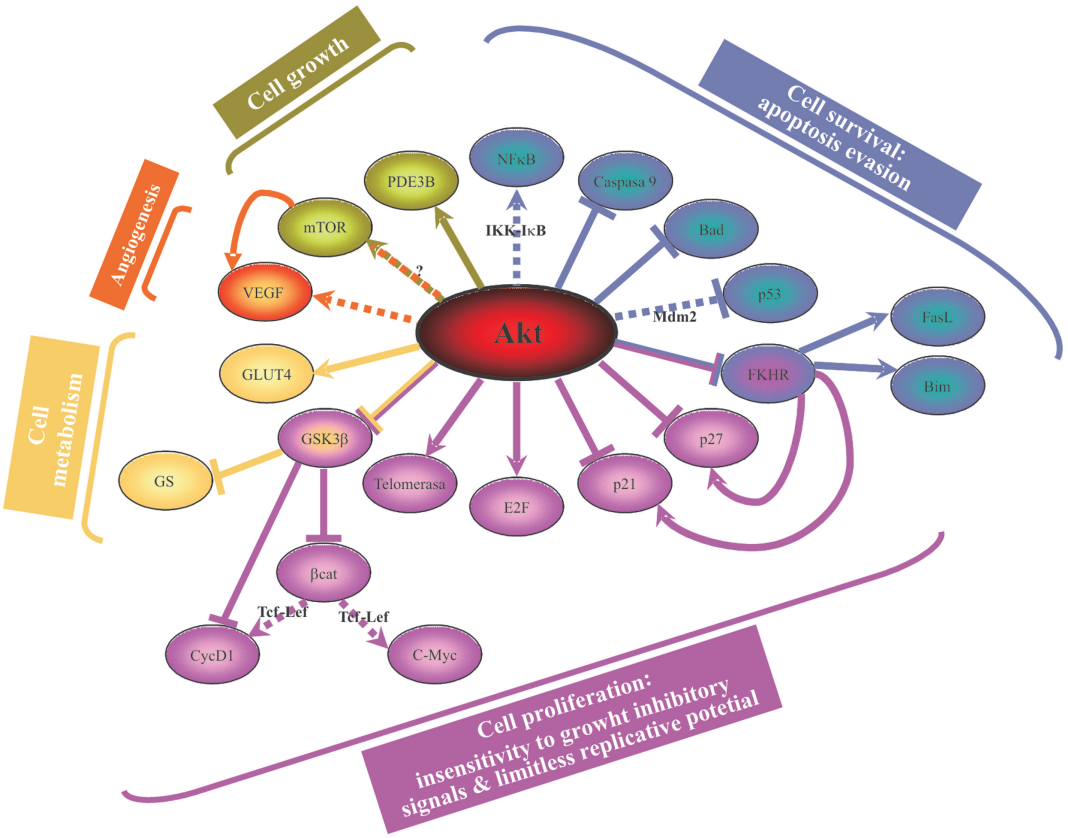

Fig. 2. Cellular functions of Akt. Many of Akt's substrates have functions that lead to cellular transformation: according to the hypothesis proposed by Hanahan and Weinberg (see main text), they represent a growth advantage to the cell, in this case in terms of inhibiting apoptosis, growing independently of growth signals from neighbouring cells, dividing continously and promoting new blood vessel formation. Other substrates participate in different cellular processes that are not directly involved in transformation but are nonetheless necessary for it.

Akt signaling pathway alterations in human cancer

Not only Akt, but also its upstream regulators and its downstream targets, have been found altered in multiple human tumors, with the net result of activated PI3K/Akt pathway. For example, increased Akt has been found to be a consequence of HER2/neu over-expression in breast tumors positive for this receptor (Bacus et al., 2002); PI3K amplification and over-activation have been detected in around 40% of ovarian and head and neck tumors (Shayesteh et al., 1999; Yuan et al., 2000; Pedrero et al., 2005), and PTEN mutation and/or inactivation has been found in 35% of endometrial cancers (Risinger et al., 1997; Terakawa et al., 2003). As to Akt targets, mTOR signaling has been found over-activated in renal tumors through inactivation of TSC2 (Kenerson et al., 2002) and in gastrointestinal polyps through disruption of LKB1 (Shaw et al., 2004).

Models of AKT function

Given the importance of Akt in cancer, much research has been done on it and various models focused on understanding its role in tumorigenesis, both cellular and animal, have been developed; we shall highlight here some of the more interesting.

Cellular models

Several groups have tested the capacity of Akt to transform cells when over-expressed. Ahmed et al. first showed that, in contrast to v-Akt-expressing cells, cells expressing c-Akt that were intraperitoneally injected into nude mice failed to induce tumors (Ahmed et al., 1993). Later on it was shown that a mutant constitutively active Akt protein that does not bind to the membrane has low or null transforming capacity, similarly to a membrane targeted kinase-defective mutant, whereas membrane-targeted forms of Akt were highly oncogenic (Aoki et al., 1998; Sun et al., 2001b). More recently, we observed that papilloma-derived PB keratinocytes overexpressing a wild type form of Akt become fully transformed, and induce highly aggressive tumors in nude mice, which progress in parallel to increasing kinase activity (Leis et al., 2002; Segrelles et al., 2002, 2004, 2006), thus suggesting that increased Akt expression can be tumorigenic upon activation by endogenous mechanisms (Leis et al., 2002; Segrelles et al., 2002, 2004, 2006). In addition, over-expression of Akt has been shown to induce resistance to chemotherapeutic agents, such as paclitaxel, a widely used antitumoral drug. The mechanism seems to be mediated by the phosphorylation and inhibition of Bad, eventually leading to decreased apoptosis (Page et al., 2000).

Mouse models

Several mouse models for Akt have been generated, both through loss-of-function and over-expression approaches (Scheid and Woodgett, 2003; Bellacosa et al., 2004; Yang et al., 2004). The former, besides indicating that there is a certain degree of functional redundancy, give information on the role of Akt proteins in development (Peng et al., 2003; Song et al., 2005; Dummler et al., 2006; Heron-Milhavet et al., 2006). Interestingly, loss of function models not only highlight the role of Akt in developmental processes, but they also show that a finely tuned regulation is required. In this regard, we have recently found that constitutive Akt activity, above a certain threshold, alters development of ectoderm-derived organs through altered BMP signaling, and it expands the adult stem cell population in epidermis (Segrelles et al., 2008). However, most mouse models showing constitutive activation or over expression of Akt in mice hint at the importance of Akt in proliferation processes. Thus, expression of constitutively activated Akt1 (by means of myristoylation sequences-MyrAkt1) in the thymus causes lymphomas with a short latency, whereas expression of AktE40K (a mutant Akt whose PH domain displays increased affinity for phospholipids) leads to the development of lymphomas in peripheral organs later in life (Malstrom et al., 2001). MyrAkt expression in prostate epithelial cells causes early tumoral lesions through activation of the p70S6K pathway (Majumder et al., 2003). In contrast, MyrAkt1 expression in mammary gland delays involution by attenuating apoptotic death, but does not induce breast tumors (Hutchinson et al., 2001). Finally, expression of MyrAkt1 or overexpression of wt Akt1 in the basal layer of stratified epithelia causes multiple spontaneous epithelial tumors and confers heightened sensitivity to chemical carcinogenesis (Segrelles et al., 2007). Overall, these studies show that Akt1 contribute to oncogenesis, probably in a tissue dependent manner, but also indicate that additional alterations are required for the cells to become fully tumorigenic.

Role of AKT in experimental tumorigenesis

The two-stage model of mouse skin chemical carcinogenesis has proved of use in the understanding of the development of squamous tumors from a molecular point of view (Yuspa, 1998). Tumors progress through three sequential steps termed initiation, progression and conversion. Initiation is an irreversible and inheritable change that does not lead to phenotypic alterations; this can be achieved through the use of dimethyl-benzanthracene (DMBA), which frequently induces mutations in the Ha-Ras oncogene. Promotion refers to the selection and expansion of the initiated population, giving rise to papillomas; this step is typically induced by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Some of these papillomas proceed through the conversion phase, forming malignant squamous cell carcinomas (Yuspa, 1998). This well established model has allowed the study of the molecular changes that underlie these sequential alterations.

Among them, Akt activity has been shown to increase during the entire process. Its activation is a consequence of increased PI3K activity at early stages, whereas ILK activation and PTEN decreased activity contribute to its sustained increased activity at later stages (Segrelles et al., 2002). In agreement, deregulated Akt activity in transgenic mice leads to heightened sensitivity to this experimental carcinogenesis protocol (Segrelles et al., 2007). Interestingly, this model displays some parallelisms with certain human tumors, including head and neck cancer (Slaga et al., 1996; Yuspa et al., 1996). Accordingly, tumors generated through injection of Akt-transfected, papilloma derived PB keratinocytes display many molecular alterations similar to those found in human HNSCC (Segrelles et al., 2006).

AKT in squamous cell carcinomas of the head and neck

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer worldwide, with roughly 540000 new cases each year; morbidity derived from the illness and its treatment is high, whereas five year survival rate is low, around 50% and without substantial improvement over two decades (Ries LAG, 2006). The term HNSCC comprises epithelial tumors that arise in the oral cavity, pharynx, larynx and nasal cavity, with the main risk factors being alcohol and/or tobacco use (up to 100 times higher for both) (Neville and Day, 2002); another important risk factor is HPV infection, which has been detected in around 20% of all cases and 50% of oropharynx cases (Gillison et al., 2000).

Numerous studies in recent years have been focused on identifying the genetic and epigenetic alterations underlying HNSCC (reviewed in (Mao et al., 2004; Lu et al., 2006)); one of the most frequently altered pathways is EGFR/Ras/PI3K, among others (p53/DNp63, pRb/CycD1, TGFß/Smad and NFκB). Alterations in EGFR itself, mainly by over-expression, have been reported in 5 to 90% of the cases; mutation and over-expression of the different Ras proteins have also been frequently detected (McDonald et al., 1994), and amplification of the PIK3CA gene (the gene coding for the catalytic unit of PI3K) has been found in around 40% of the cases (Pedrero et al., 2005). All these genetic alterations can lead to increased Akt activity, which has indeed been found in 20 to 60% of tumor samples and in the majority of HNSCC-derived cell lines (Amornphimoltham et al., 2004; Pedrero et al., 2005; Mandal et al., 2006; Segrelles et al., 2006) (Fig. 3). Besides that, Akt2 gene amplification has been found in 30% of HNSCC samples (Pedrero et al., 2005). Interestingly, tissue microarray studies showed that there is no EGFR activation in 50% of the cases in which active Akt can be found (Molinolo et al., 2007). Finally, Akt activation has been found to be predictive of poor clinical outcome, being associated with higher local recurrence rates and decreased survival (Massarelli et al., 2005; Yu et al., 2007).

AKT as a therapeutic target in HNSCC

The prominent role of Akt and its signaling pathway in the development of tumors has led to the development of drugs that might be of clinical use in the treatment of cancer through the inhibition of Akt itself or its regulators and targets.

EGFR, one of the tyrosine kinase receptors acting upstream of the Akt signaling pathway, has been successfully targeted, with different phase II and III clinical trials giving promising results. One of these

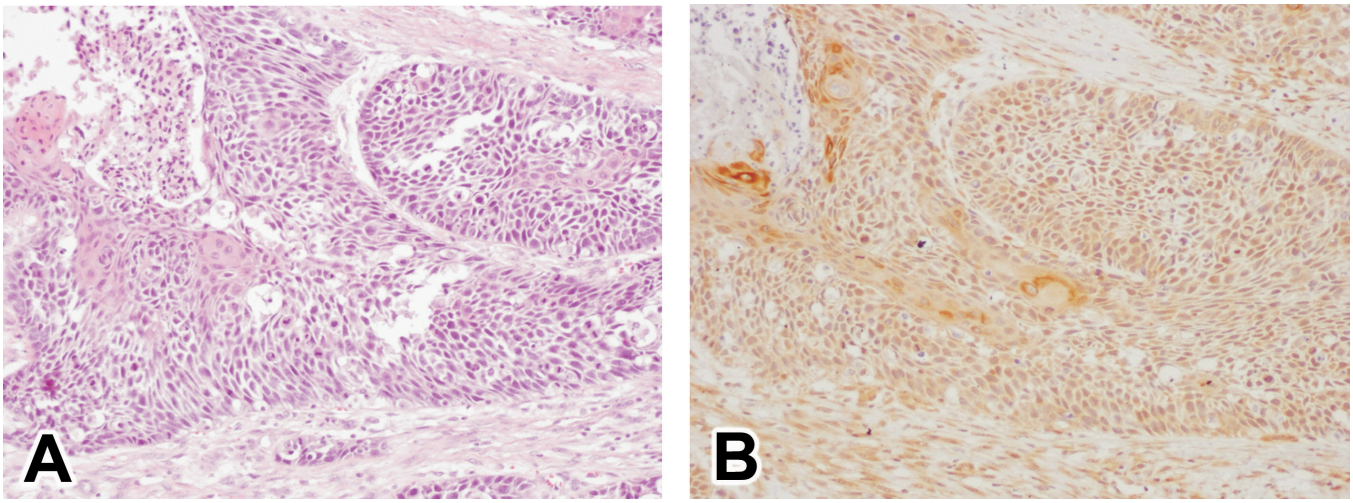

Fig. 3. Akt activation in HNSCC. Representative micrograph of a carcinoma of the head and neck **(A)** and the same section stained with an antibody that recognizes the phosphorylated, active form of Akt **(B)**. x 10

includes cetuximab, a monoclonal antibody against EGFR, in combination with radiotherapy or other chemotherapeutic agents (Bonner et al., 2006). In 2006 the FDA approved the use of cetuximab in combination with radiotherapy to treat unresectable cases of HNSCC, and its use as a single agent for patients with metastatic HNSCC previously treated with standard chemotherapy. An undesired limitation of the treatment is the reported efficiency of cetuximab only on wild type EGFR, with some common mutant forms, such as EGFRvIII, being resistant to therapy. Importantly, this mutated form is expressed, together with the wild type form, in around 40% of head and neck tumors, and contributes to enhanced tumor growth (Sok et al., 2006). Gefitinib, a small molecule tyrosine kinase inhibitor drug that targets EGFR, has not yielded such good results in clinical trials for its use as a monotherapy (Cohen et al., 2003, 2005), but it has proved of use in combination therapy with other chemotherapeutic agents or with radiation (Wirth et al., 2005; Chen et al., 2007);. Studies on HNSCC cell lines indicated that sensitivity to gefitinib associates with EGFR amplification, whereas resistance to this drug comes from ErbB3 expression and ErbB2 phosphorylation. Accordingly, treatment of resistant cell lines with gefitinib and pertuzumab, a humanized antibody that interferes with ErbB2 heterodimerization, prevents their growth (Erjala et al., 2006). In another example of the use of combination therapy, Chun et al. used gefitinib together with gemcitabine (a nucleoside analog) in a HNSCC cell line, and to treat mice bearing xenografted tumors derived from it (Chun et al., 2006). They found that gemcitabine followed by gefitinib caused arrest of cells in S-phase, decreased both EGFR and Akt phosphorylation and increased apoptosis, leading to tumor regression in the xenografted mice (Chun et al., 2006). Erlotinib, another drug belonging to the small molecule tyrosine kinase inhibitor class, also shows better results in combination therapies than in monotherapy in clinical trials (Soulieres et al., 2004; Patnaik et al., 2006; Siu et al., 2007)

UCN-01 (7-hydroxy-staurosporine) is an interesting inhibitor of PDK1, and inhibits the growth of tumor cells of various origins. Among them, it has been shown to inhibit the growth of HNSCC-derived cell lines and tumors derived from them through a G1 cell cycle arrest, mediated by decreased cyclin D3 and increased p27 expression, and through increased apoptosis (Patel et al., 2002). Posterior studies showed that UCN-01 induces a dramatic decrease both in the levels of active Akt and its targets in HNSCC cell lines (Amornphimoltham et al., 2004), suggesting that this drug might be of potential use in the treatment of HNSCCs. However, although several clinical trials in phase I have already been done, with some favorable results, for UCN-01 alone and in combination with other drugs, none of them addresses its use in HNSCC.

Recently, the small-molecule kinase inhibitor KP372-1 has been tested with promising results in HNSCC-derived cell lines. Treatment of two representative cell lines with KP372-1 blocked Akt activation and phosphorylation of its downstream target S6, inhibited proliferation and induced apoptosis and anoikis (Mandal et al., 2006). Similar results were obtained when other tumor cell lines were used (Koul et al., 2006; Zeng et al., 2006). However, despite these encouraging results, no clinical or even preclinical trials have been performed so far with this inhibitor.

The Akt's downstream target mTOR is one of the most widely studied in terms of clinical development of anticancer agents, due to it functions mediating many of Akt's functions. Rapamycin, which inhibits mTOR, prevents accumulation of S6 and, to a minor extent, 4E-BP1 in a large panel of HNSCC cell lines, as well as in tumor xenografts. In these, treatment with rapamycin led to tumor remission through a rapid but transient induction of apoptosis and a sustained inhibition of the proliferative capacity of the remaining tumor cells, together with a reduction in tumor vascularity (Amornphimoltham et al., 2005). Rapamycin and other rapamycin-derived inhibitors of mTOR, such as CCI-779 and RAD001, have been proved of use, either alone or in combination with other chemotherapeutic agents, in preclinical models of other tumor types for which a role for Akt has been shown (deGraffenried et al., 2004; Majumder et al., 2004). However, phase I and II clinical trials performed provide only modest results (low partial response or slight tumor regression) (Chan et al., 2005; Galanis et al., 2005; Yee et al., 2006). Importantly, the study by Galanis et al. showed a better response to the drug when tumors also showed high levels of phosphorylated p70S6K, the kinase that phosphorylates S6. The poor responses to rapamycin are intriguing and surprising. The possible explanation might be that mTOR inhibition prevents phosphorylation and inhibition of IRS, which would lead to PI3K and Akt activation (Shi et al., 2005; Sun et al., 2005; O'Reilly et al., 2006). Nonetheless, these studies also indicated that Akt activation is highly dependent on IGF signaling. Accordingly, the use of IGF-1R or PI3K inhibitors enhances the antiproliferative effects of rapamycin. This points to the possible advantageous use of combining an mTOR inhibitor with a PI3K/Akt inhibitor. To date, this feedback loop has not been reported in HNSCC, but, since IGF-R1 over-expression has been detected in oral tumors (Brady et al., 2007), it is possible that it exists and thus combination therapy would also be advisable, if not required, in this case. Also, using tissue microarray techniques, Molinolo et al. proved the existence of a subgroup of HNSCC patients characterized by mTOR activation through an alternative activation of Akt (phosphorylated in Ser473 but not in Thr308) (Molinolo et al., 2007). In agreement, a preclinical study with CCI-779 and erlotinib in a HNSCC cell line shows that this combination results in a synergistic effect, reducing Akt and p70 activation in cell culture as well as tumor xenograft growth and angiogenesis *in vivo* (Jimeno et al., 2007).

A novel approach using antisense oligonucleotides

directed against eIF4E can also be promising as antitumoral therapy. Increased eIF4E levels are a common hallmark of head and neck cancer and other tumors and promote augmented translation of specific mRNAs. Remarkably, eIF4E favors the translation of cyclin D1, c-Myc, Bcl-2 and VEGF among others; thus, its over-expression has a clear influence on cell growth and survival and tumor angiogenesis (reviewed in (De Benedetti and Graff, 2004)). In a recent study, Graff et al. used antisense oligonucleotides against eIF4E and demonstrated that reduction of eIF4E levels induces apoptosis and suppresses tumor growth, reduces the expression of the above mentioned pro-tumorigenic proteins and has an antiangiogenic effect (Graff et al., 2007).

Concluding remarks

Akt/PKB plays a prominent role in tumorigenic processes in multiple cancer types, including head and neck tumors. Accordingly, many current approaches in treating these types of tumors target this kinase and its pathway, yielding excellent results particularly in combination therapy. Nonetheless more research is still required, as cellular transformation depends on multiple interactions and complex pathways, which provide unexpected results; this is underscored by rapamycin, which, besides inhibiting mTOR, indirectly activates Akt. Additionally, targeted therapies should also take into account the specific molecular alterations found in each tumor, since they might modify the outcome of the treatment, as is best exemplified by the low efficiency of cetuximab on mutant EGFR. Consequently, one might argue that future treatments for HNSCC will rely on three facts: 1) having a deep knowledge of all the effects that each specific drug has on tumor cells; 2) targeting several pathways –or different branches of the same pathway– simultaneously, probably including PI3K/Akt pathway; and 3) characterizing molecularly every case so that the best drug combination can be chosen.

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