The importance of the PI3K/AKT/mTOR signaling pathway in canine neoplasms: Literature review

La importancia de la vía PI3K/AKT/mTOR de señalización en las neoplasias caninas: revisión de literatura

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Abstract. The PI3K/AKT/mTOR pathway is related to proliferation, protein synthesis, survival, angiogenesis, apoptosis, and cell motility. Genetic alterations in either activation of oncogenes or inactivation of tumor suppressor make it the second most altered pathway in neoplastic processes. The PI3K/AKT/mTOR pathway is currently considered an attractive target for the development of anti-tumor molecules. Specific inhibitors of this pathway are under development, and those already recognized are being tested in clinical trials, representing a promising approach for the treatment of cancer patients. It is believed that, as this pathway is involved in the development of many human cancers, its activation may also be related to the development of various canine neoplasms. Therefore, this review aims to describe the state-of-the-art knowledge about the PI3K/AKT/mTOR pathway and highlight some research performed with either canine tumors or cellular lines.

Key words: mast cell tumors, osteosarcoma, hemangiosarcoma, mammary carcinoma, dog.

INTRODUCTION

Currently, numerous studies have been conducted in both human and veterinary medicine, aiming to unravel the mechanisms that may be involved in the etiology of cancer. This knowledge will lead to the development of new anti-tumor molecules, or even, help select patients with a particular cancer, to undergo therapies already used in patients with other malignancies known to respond to that specific therapy (Macauliffe et al 2010, Hanahan and Weinberg 2011, Chen et al 2012).

In recent years the PI3K/AKT/mTOR (phosphatidylinositol 3 kinase/kinase B protein/rapamycin target in mammals) signaling pathway has attracted the attention of many researchers because it is known that it is implicated in the etiology of various neoplasms. Furthermore, some of the components of this pathway present specific inhibitors that are currently in clinical trial stages (Macauliffe et al 2010, Ghayad and Cohen 2010).

Thus, this review describes the PI3K/AKT/mTOR signaling pathway and cites some studies in veterinary medicine performed to assess the expression and efficacy of specific inhibitors in cellular lines or tumors of dogs.

THE PI3K/AKT/mTOR PATHWAY

Hanahan and Weinberg (2011) described ten cellular changes related to malignant transformation of neoplastic cells: genomic instability and mutation, evasion of apoptosis, limitless replicative potential, insensitivity to inhibition of growth factors, continuous angiogenesis, invasion mechanisms and cellular of metastasis, self-sufficiency of growth factors, reorganization of energy metabolism, and evasion of cell destruction by the immune system.

In all these systems, the kinase proteins, when abnormally activated, act on the cell cycle regulation, metabolism, on cell motility, the response to the microenvironment, DNA damage repair and apoptosis. Some studies suggest that these proteins are commonly activated in cancer cells, contributing to cell proliferation and carcinogenesis (Dancey and Sausville 2003, Dillon et al 2007, Jiang and Liu 2008).

The PI3K/AKT/mTOR signaling pathway comprises a cascade of serine/threonine kinases that regulate a variety...
of cellular processes, including cell cycle progression, cell survival, migration and protein synthesis. Recent evidence has proven that the deregulation of this pathway is associated with promoting tumorigenesis and angiogenesis in various cancers (Jiang and Liu 2009).

Activation of tyrosine-kinase receptors induces the activation of the PI3K/AKT/mTOR pathway (Knowlden et al 2008). Once the receptor is activated, the intracellular portion thereof is autophosphorylated and is used as "docking site" for some proteins like PI3K (Marone et al 2008). Once phosphorylated, the PI3K is responsible for the conversion of PIP2 (phosphatidylinositol-4,5-biphosphate) into PIP3 (phosphatidylinositol-3,4,5-triphosphate), thereby recruiting protein that contain homology by pleckistrina (PH), such as AKT and PDK1 (protein-kinase 1 dependent on phospholipase-3) (Martelli et al 2007, Kang et al 2005). The interaction between the PH domain of AKT and PIP3 promotes conformational changes in the AKT molecule, resulting in the exposure of two phosphorylation sites (Thr 308 in the kinase domain and Ser 473 in the regulatory domain) (Jacinto et al 2006).

The PTEN (phosphatase and deleted homologous tensin on chromosome 10) is a tumor suppressor gene, responsible for dephosphorylating PIP3 into PIP2. However, the loss or decreased expression of PTEN indirectly stimulates the activity of PI3K, leading to constitutive activation of AKT and upregulation of mTOR (Mc Auliffe et al 2010).

Two main events are responsible for full activation of AKT: in the first, AKT is partially activated by phosphorylation of Thr 308 by PDK1; in the second, full activation of AKT requires phosphorylation of Ser 473 by mTOR-Rictor (Efeyan and Sabatini 2010).

The regulation of mTOR by the AKT protein may occur directly or indirectly (Laplante and Sabatini 2009). In the first case, AKT protein can activate mTOR by phosphorylation of Thr 2446 and Ser 2448 protein do-

mTOR bound to Rictor (protein associated with TOR, insensitive to rapamycin), GβL and mSin 1 (protein kinase associated with activated mitogenic protein 1) (Laplante and Sabatini 2009). Therefore, the activation of 4EBP1 is often used as a marker of TORC1 activity (figure 1) (Hay and Sonenberg 2004).

The TORC2 complex controls the cytoskeleton actin, in addition to being capable of phosphorylating AKT;
therefore, some studies suggest that this protein complex corresponds to PDK2, hitherto unknown and responsible for a positive feedback pathway (Foster and Fingar 2010).

The assessment of the PI3K/AKT/mTOR pathway and its aberrant activation have been described in several human cancers, such as breast cancer, colorectal cancer, squamous cell carcinomas, angiosarcomas and soft tissue tumors (Castaneda et al. 2010, Quesnelle et al. 2007, Clark et al. 2010, Lahat et al. 2010, Dobashi et al. 2009). After p53, the PI3K/AKT/mTOR is the most frequently activated pathway in neoplastic processes (Agarwal et al. 2010).

The interest in the components of this pathway has been growing continuously since these are currently considered promising targets for the development of new anticancer molecules. Some of these inhibitors, such as NVP-BKM120, PI3K inhibitor; Perifosine and Triciribine, AKT inhibitors; Temsirolimus, Rapamycin and Everolimus, mTOR inhibitors; and NVP-BEZ235, dual inhibitor of PI3K and mTOR are being used in clinical trials (Ghayad and Cohen 2010).

**THE PI3K/AKT/MTOR PATHWAY IN CANINE TUMORS**

In veterinary medicine, the activation of this pathway has been extensively studied in canine neoplasms. Some studies were conducted with canine cell lines while others were performed with neoplastic tissues of patients routinely treated in veterinary hospitals. Some of these studies also evaluated the efficacy of specific inhibitors on the studied materials (Chen et al. 2012, Gordon et al. 2008, Qiu et al. 2008a,b, Kent et al. 2009, Paolini et al. 2010, Murai et al. 2012, Rodriguez et al. 2012).

**OSTEOSARCOMA**

The activation of this pathway in canine osteosarcoma cell lines was evaluated by the Western blot technique, in which the presence of both total and phosphorylated mTOR and S6K1 were assessed before and after the cells were exposed to Rapamycin. The cells were also subjected to a clonogenic assay (to assess the ability of colony formation) before and after their exposure to the same drug. This study demonstrated the pathway activation for all studied lines and showed that Rapamycin was able to inhibit mTOR activity. The clonogenic assay demonstrated how effectively this inhibitor reduces survival of tumor cells exposed to the drug, suggesting that this can have a beneficial effect on canine osteosarcoma cells (Gordon et al. 2008).

A clinical trial was performed using 22 dogs with osteosarcoma that were treated with Rapamycin in doses ranging from 0.01 to 0.08 mg/kg, every 24 hours for 7 days. The animals underwent an incisional biopsy at diagnosis and then, again, within seven days of Rapamycin daily applications. After this period, the animal affected limb was amputated. Tumors from biopsy and amputation were subjected to the electrochemiluminescence technique to detect total and phosphorylated AKT and S6K1. The

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phosphorylated/total protein ratio (p-S6K1/S6K1) decreased in the samples treated with the inhibitor, thus showing that Rapamycin is able to modulate the pathway. In this experiment, the drug adverse effects were also evaluated, and it was observed that they were not associated with the used dosage. Therefore, this experiment suggests that Rapamycin may be administered safely and can produce therapeutic concentrations in dogs with osteosarcoma (Paolini et al 2010).

MELANOMA

Expression of total and phosphorylated AKT, mTOR, and S6K1 was evaluated in cell lines of canine oral melanoma using the Western blot technique before and after the cells were exposed to Rapamycin. Similarly, the cells were subjected to a clonogenic assay to evaluate the survival fraction before and after exposure to mTOR inhibitor. This study showed that AKT, mTOR and its effector, S6K1, were present and active in cell lines of canine oral melanoma. It also demonstrated that Rapamycin was capable of inhibiting mTOR activity, as well as reducing the survival fraction of the studied cell lines, suggesting that this inhibitor have a beneficial effect on canine oral melanoma cells (Kent et al 2009).

HEMANGIOSARCOMA

The immunohistochemical technique was used to assess the expression of the phosphorylated proteins AKT Thr 308, AKT Ser 473, mTOR Ser 2448, 4EBP1 Thr 37/46, and eIF4E by Western blot technique. The detectable levels of phosphorylated proteins demonstrated the pathway activation in these cell lines. In this same study, all cell lines were exposed to inhibitors specific to PI3K, AKT and mTOR, which resulted in viability inhibition. The authors also evaluated the efficacy of the combination of inhibitors of PI3K and mTOR in all cell lines. The association between the pathway inhibitors (PI3K, AKT and mTOR) and doxorubicin chemotherapy was evaluated in hemangiosarcoma and breast carcinoma cell lines. The trial results showed that the combination of the two inhibitors resulted in a synergistic effect on the glioma cell line and an additive effect on the other cell lines evaluated. However, the association of the PI3K inhibitor to chemotherapy showed antagonistic effect for the two cell lines evaluated and the association of AKT inhibitor to doxorubicin resulted in synergistic effect in the hemangiosarcoma cell line and antagonistic effect in breast carcinoma. However, the combination of mTOR inhibitor and doxorubicin resulted in an additive effect in both cell lines evaluated. The authors commented on the importance of the pathway in these canine cell lines, highlighting potential therapeutic targets within this pathway (Chen et al 2012).

VARIOUS CELL LINES

Chen et al (2012) used five different canine cell lines from B-cell lymphoma, mammary carcinoma, hemangiosarcoma, mast cell tumor, and glioma, to evaluate the expression of the phosphorylated proteins AKT, mTOR, S6RP, 4EBP1 and eIF4E by Western blot technique. The detectable levels of phosphorylated proteins demonstrated the pathway activation in these cell lines. In this same study, all cell lines were exposed to inhibitors specific to PI3K, AKT and mTOR, which resulted in viability inhibition. The authors also evaluated the efficacy of the combination of inhibitors of PI3K and mTOR in all cell lines. The association between the pathway inhibitors (PI3K, AKT and mTOR) and doxorubicin chemotherapy was evaluated in hemangiosarcoma and breast carcinoma cell lines. The trial results showed that the combination of the two inhibitors resulted in a synergistic effect on the glioma cell line and an additive effect on the other cell lines evaluated. However, the association of the PI3K inhibitor to chemotherapy showed antagonistic effect for the two cell lines evaluated and the association of AKT inhibitor to doxorubicin resulted in synergistic effect in the hemangiosarcoma cell line and antagonistic effect in breast carcinoma. However, the combination of mTOR inhibitor and doxorubicin resulted in an additive effect in both cell lines evaluated. The authors commented on the importance of the pathway in these canine cell lines, highlighting potential therapeutic targets within this pathway (Chen et al 2012).

FINAL CONSIDERATIONS

Treatment of cancer patients in veterinary medicine is often a challenge, since many patients still do not have a satisfactory response to therapy. Therefore, research on new therapeutic possibilities with fewer adverse effects
and better results are desirable in veterinary oncology, given the increasing number of cancer patients in small animal clinic.

As well as the human medicine model, where this pathway have been used routinely in several types of cancer due its huge importance in terms of more individual therapeutic target, the evaluation and knowledge of this pathway by veterinarians, becomes a very interesting possibility in the future, since early studies of this pathway have shown promising results to treat neoplastic disorders in small animals.

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