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The Akt inhibitor KP372-1 inhibits proliferation and induces apoptosis and anoikis in squamous cell carcinoma of the head and neck

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Summary Therapies that target signaling pathways critical to the pathogenesis and progression of squamous cell carcinoma of the head and neck (HNSCC) are needed. One such target, phosphatidylinositol 3-kinase, and its downstream target serine/threonine kinase, Akt, are up-regulated in HNSCC. Targeted therapy could consist of inhibitors of these kinases or, alternatively, of inhibitors of the pathways that they regulate. To explore the effect of Akt inhibition on the growth and survival of HNSCC tumors, we evaluated the effect of a novel Akt inhibitor, KP372-1, on the growth, survival, and sensitivity to anoikis of HNSCC cell lines in culture. Using Western blotting of head and neck cancer cell lines and squamous mucosa

Abbreviations: EGF: epidermal growth factor; GSK-3: glycogen synthase kinase-3; HNSCC: squamous cell carcinomas of the head and neck; MTT: thiazolyl blue tetrazolium bromide; p: phosphorylated; PARP: poly (ADP-ribose) polymerase; PI-3K: phosphatidylinositol 3-kinase; SCC: squamous cell carcinoma.

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and carcinoma specimens, we found that Akt was highly phosphorylated in head and neck cancer cell lines and human head and neck squamous carcinoma specimens. Treatment of HNSCC cell lines with KP372-1 blocked the activation of Akt, inhibited head and neck cancer cell proliferation, and induced apoptosis and anoikis in several HNSCC cell lines. Furthermore, KP372-1 decreased the phosphorylation of the S6 ribosomal (Ser240/244) protein, which is a downstream target of Akt. Taken together, these findings indicate that KP372-1 may be a useful therapeutic agent for HNSCC and should be further evaluated in preclinical models of HNSCC.

Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is one of the leading causes of cancer deaths worldwide.[1] Despite improvements in surgery, radiotherapy, and conventional chemotherapy, patients at high risk for tumor recurrence, according to clinicopathologic criteria, frequently develop locoregional recurrences, leading to 5-year survival rates that remain at only 30—50%.[2] Although nodal metastases and extension of the tumor beyond the lymph node capsule have long been known as the most predictive factors for regional recurrence and death from HNSCC, little is known about the biologic basis of tumor progression of HNSCC.[3] Therefore, there is a great need to identify the signaling pathways important in the development and progression of HNSCC.

The phosphatidylinositol-3 kinase (PI-3K)/Akt signaling pathway has been shown to mediate tumor cell proliferation and survival and to be up-regulated in a number of tumor types, including HNSCC.[4—7] Others have shown that higher levels of Akt expression contribute to malignancy and antiapoptotic activity in squamous cell carcinoma (SCC) of oral squamous epithelium.[8] The Akt pathway has also been shown to be important in mediating signals that lead to anchorage-independent survival, and inhibition of the PI-3K/Akt has been shown in other cell types to lead to anchorage-independent cell death, or anoikis.[9,10] Our studies in which oral cavity cancer cells were selected for anchorage-independent survival support the findings of Li et al.[11] that selection for resistance to anoikis leads to aggressive tumor growth and decreased animal survival in an orthotopic model of tongue cancer in nude mice. Given the association between anoikis resistance and the tumor progression of oral SCC and the associations between anoikis resistance and the PI-3K/Akt pathway, we evaluated the effects of a specific Akt inhibitor, KP372-1 (molecular weight 224.2; QLT Inc., Vancouver, BC, Canada), on the inhibition of PI-3K/Akt pathways biochemically and on cell proliferation, apoptosis, and anoikis in head and neck cancer cell lines.

Materials and methods

Cell cultures and reagents

The Tu167, Tu212, Tu159, LN212, UMSCC1, and MDA1986 HNSCC cell lines were obtained from Dr. Gary Clayman at The University of Texas M.D. Anderson Cancer Center Head and Neck Laboratory. All cell lines were maintained in Dulbecco's modified Eagle's/F-12 medium supplemented with 10% fetal calf serum. For the selection of anoikis-resistant cells, 2×10^6 Tu167 cells were detached from a tissue-culture flask by treatment with 0.25% trypsin—0.1% ethylenediaminetetraacetic acid solution and then grown in a 15-ml conical tube (Falcon; Becton-Dickinson, Franklin Lakes, NJ) with a vented cap (Biocoat; Becton-Dickinson, Bedford, MA) that was placed on a rotating wheel for 72 h in a humidified incubator at 37 °C with 5% CO_2. These cells were then plated and grown to confluence under adherent conditions before they were expanded. To generate the JMAR cell line from the Tu167 cell line, this cycle was repeated six times. Limiting dilution cloning of the Tu167 and JMAR cell lines was performed, yielding the cell lines Tu167c2, JMARc39, and JMARc42.[12] In addition, DM12, a clone of Tu167, was selected for its ability to grow in soft agar.

The following antibodies, reagents, and materials were used: phospho (p) Akt/Ser473, pAkt/Thr308, total Akt, p70S6 kinase, S6 ribosomal protein, pGSK-β antibodies, and an Akt kinase assay kit (all from Cell Signaling Technology, Beverly, MA); anti-actin (Sigma, St. Louis, MO); anti-mouse and anti-rabbit horseradish-peroxidase conjugate (Amersham, Piscataway, NJ); poly (ADP-ribose) polymerase and p85 (Upstate Biotechnology, Lake