REVIEW ARTICLE

Mechanism and Potential Therapy in Ameloblastoma: Akt Signaling Pathway

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Received date: Feb 8, 2022; Revised date: Mar 1, 2022; Accepted date: Mar 1, 2022

Abstract

BACKGROUND: Ameloblastoma is the most common benign aggressive tumor. They are more prevalent in the mandible than in the maxilla, mostly observed on the posterior of the jaw. Ameloblastoma can arise at any age, however it most usually affect patients between the ages of 20 and 40. Numerous efforts have been made to develop molecular targeted therapies to treat cancers, such as Akt inhibitors. However, these drugs have not been tested for treating ameloblastoma yet, since underlying molecular factors have yet to be identified. This study was carried out to delineate possible molecular mechanisms related to the Akt signaling pathway in ameloblastoma and potential drugs for ameloblastoma treatment.

CONTENT: Akt signaling pathway in ameloblastoma has been implicated in the formation and progression of tumors. Akt signaling is involved in various cellular mechanisms, such as cell cycle, apoptosis, and cytoskeletal rearrangement, which includes Phosphatidyl Inositol 3 Kinase (PI3K)-Akt signaling, Akt-Nuclear Factor (NF)-κB signaling, Akt-Mammalian Target of Rapamycin (mTOR) signaling, Akt-B-cell Lymphoma (Bcl)-2 Family signaling, Akt-Survivin signaling. Potential ways of treatments using chemical compounds and micro RNA (miRNA), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were explored as well.

SUMMARY: The present review highlights various Akt signaling involved in ameloblastoma and its potential pathways for treatments, while the gold standard of ameloblastoma treatment is still surgery to remove the tumor, there are many potential agents through various means of inhibition for ameloblastoma. Therefore, understanding the underlying signaling on ameloblastoma is necessary to induce inhibition on ameloblastoma. More research in potential ways to inhibit Akt signaling in ameloblastoma will lead to a better management of ameloblastoma in the future.

KEYWORDS: ameloblastoma, Akt, PI3K, NFκB, mTOR, Bcl-2, miRNA, CRISPR

Indones Biomed J. 2022; 14(1): 1-10

Introduction

Maxillofacial tumors can be classified according to their origins, as odontogenic or non-odontogenic.(1) In general, odontogenic tumors are rare lesions, ranging from 0.7 to 2.5% among all biopsy specimens.(2) Among all odontogenic tumors, ameloblastoma is the most common benign aggressive tumor, representing 40% of odontogenic tumors, followed by odontoma (20%) and odontogenic keratocyst (14%).(1,2) Ameloblastoma is more prevalent in the mandible than in the maxilla, mostly observed on the posterior of the jaw. Ameloblastoma can arise at any age, however it usually affect people between the ages of 20 and 40. It is rare in children under the age of 10, and there is no significant difference in the number of affected males and females.(3)

Ameloblastoma typically occurs within the third and fourth decade of life, with an equal incidence rate in men and women.(1,4) Patients typically present with slowly painless

enlarging facial swelling, however most of them found incidentally on radiographic evaluation.(1,5) Although benign and slow-growing in nature, these neoplasms are locally destructive, especially to local tissues like bone, and the recurrence rate is high if not adequately excised.(1,4)

Over the past few decades, many research groups have conducted investigations in the molecular biology of ameloblastoma, and the understanding of this tumor has been improved. While many aspects of local invasiveness in ameloblastoma remain unknown, it may be associated to the rate of cellular proliferation.(6) It is well established that the Akt signaling pathway, also known as Protein Kinase B (PKB), which plays a key role in the incidence and development of tumors and is involved in a variety of cellular activities including proliferation, apoptosis, cell cycle, and cytoskeletal rearrangement of tumors.(6-8) Additionally, studies have shown a relationship between Akt activation and cancer growth and pathological processes, such as invasion extent and depth, lymph node metastasis, and pathological stage.(7) While several mechanisms of the Akt signaling pathway associated with ameloblastoma are clarified, many of them remain unexplored.

Numerous efforts have been made to develop molecular targeted therapies, such as Akt or phosphatidylinositol 3 kinase (PI3K) inhibitors, to treat cancers. However, these drugs have not been tested for treating ameloblastoma yet, since underlying molecular factors have yet to be identified. Therefore, this study was carried out to delineate possible molecular mechanisms related to the Akt signaling pathway in ameloblastoma and potential drugs for ameloblastoma treatment.

Cellular Signaling and Akt

Cell signaling begins with the binding of ligands to receptors, which are complementary structures to transmembrane proteins. Ligand binding causes receptor alterations and a series of responses performed by a second messenger that transforms the receptor's message into a measurable effector function. As a result, cell signaling is essential to the cellular response system.(9) Domains are characteristically folded parts of proteins that give them specific functions and allow them to participate in signaling pathways. Proteins with comparable activities but distinct domains exist, and vice versa. Domain lengths vary from 50 to 300 amino acid residues. Domains regulate the functional units of proteins. In addition, domains can be modified by genetically engineering proteins to create mutants. Recombinant events cause domain shuffling, resulting in proteins with different domain configurations, further diversifying protein function.(10)

Signals or ligands are one of the main components in cell signaling. Cells are typically stimulated mechanically, electrically, or chemically. Most of the cell signals are chemical in nature. Growth factors, hormones, cytokines, neurotransmitters, and components of the extracellular matrix, among others, may induce eukaryotic cells to react.(11) Signaling can be endocrine, paracrine, juxtacrine, autocrine, or neuronal-neurotransmitter mediated. There are different chemical components of the ligands, including lipids, proteins, sugar polymers, nucleic acids, etc.(9)

There are two different types of receptors, which are cell-surface and intracellular receptors. The cell-surface receptors have an extracellular ligand-binding domain, a hydrophobic transmembrane region, and a cytoplasmic domain that span the plasma membrane. When ligands bind to the extracellular membrane-spanning receptors, they change their extracellular domain and activate the enzyme system associated with the cytoplasmic domain, which normally consists of kinases, phosphatases, and adaptors. G-protein, tyrosine kinases and ionotropic receptors are the three types of cell surface receptors. Intracellular receptors may be nuclear, cytoplasmic, and organellar receptors, which can be found in the endoplasmic reticulum, mitochondria, and Golgi apparatus.(12) Receptors react to ligands with high binding affinity; for example, the insulin receptor, which has a high binding affinity for just insulin. Different cell types may have varying numbers and kinds of receptors, with some lacking certain receptors while others have an excess of a particular type. Rarely, receptors involved in signal detection may cluster on the surface of cells to improve responsiveness.(13)

Src homology domain (SH)3 is a frequently seen domain in cells that consists of short residues that facilitate protein interaction. The SH3 domain is composed of five to eight -strands structured in two antiparallel -sheets or a -barrel; these strands are critical for identifying certain binding partners. The SH3 domains are found in several proteins that regulate cytoskeletal changes, PI3K, Ras Guanosine-5'-Triphosphate (GTP)ase-activating proteins, Cell Division Control protein 24 (CDC24), myosin, and phospholipase C. SH2, a phosphotyrosine-specific recognition domain, interacts with a subset of tyrosine residues that have been phosphorylated by the catalytic activity of kinases.(9) Pleckstrin homology (PH) domain is also often seen in proteins that serve as cytoskeletal components. PH domain has an affinity for Phosphatidylinositol-(3,4,5)-

trisphosphate (PIP_3) and Phosphatidylinositol-(4,5)-bisphosphonate (PIP_2). PIP_2 is necessary for phospholipase D and Adenosine Diphosphate (ADP) ribosylation factors (ARF) function. PH domain-containing proteins include Ser/Thr kinases like the Akt/Rac family.(14) The most prevalent protein identified in eukaryotic DNA-binding proteins is the leucine zipper (bZIP) domain. Immunoglobulin-like domains are typically present in proteins belonging to the immunoglobulin superfamily and are involved in cellular adhesion, activation, and molecular interaction.(15)

Akt is one of the most essential signaling molecules in cancer.(6) Akt is a protein kinase that belongs to the AGC family. AGC family is named after the protein kinase A, G, and C families, including cyclic AMP, Adenosine 3',5'-Cyclic Monophosphate (cAMP)-dependent protein kinase (PKA)/cyclic Guanosine Monophosphate (cGMP)-dependent Protein Kinase (PKG)/Protein Kinase C (PKC). In general, there are three isoforms of Akt, which are Akt1, Akt2, and Akt3 (Figure 1). The Akt1 isoform is primarily engaged in cell survival and apoptosis, whereas the Akt2 isoform modulates tumor metabolism, cell invasion, migration, metabolism, and cell death via the intrinsic mitochondrial route, and the Akt3 isoform is related with tumor cell migration.(6,16)

The PI3K/Akt signaling system is often dysregulated in malignancies and has recently emerged as a significant therapeutic target. By activating downstream effector molecules involved in the cell cycle, growth, and proliferation, the PI3K/Akt signaling pathway controls various cellular physiological functions. This is a typical

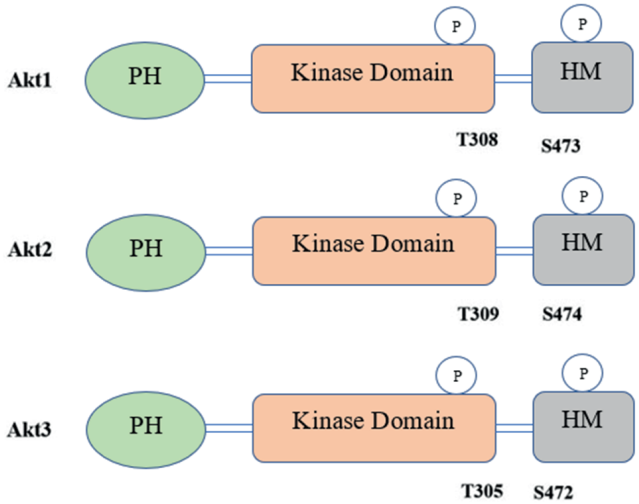

Figure 1. Schematic representation of the three Akt isoforms. Akt kinases shows similar structural organization, with an N-terminal PH domain, a central catalytic domain and a C-terminal hydrophobic motif (HM), connected by hinge region.

occurrence; overactivation of the system has been associated to cancer development in human malignancies.(17) In this review, we will discuss Akt pathway signaling related to ameloblastoma and related potential treatment approaches.

Akt Signaling in Ameloblastoma

The mechanisms underlying ameloblastoma's local invasiveness is still unknown, although their genes and signaling pathways, including Patch, Sonic Hedgehog (SHH), Smoothened (SMO), Akt, mTOR (mammalian target of rapamycin), and others, were reported to be involved in the pathogenesis.(6,18) Tumor biology can take on two distinct forms, with antiapoptotic proliferating sites on the periphery and proapoptotic differentiation sites in the center.(19) The tumor's peripheral cells that have chosen cell survival mode are believed to be the cause of disease progression that results in an expansile jaw lesion. Survivin, B-cell lymphoma (Bcl)-2, and Bcl-X were found more frequently in ameloblastoma outer layer cells, although pro-apoptotic molecules such as Fas, Fas Ligand (FasL), and Caspase-3 are found in central stellate reticulum-like cells. (19-21)

Akt is a 480 amino protein with a PH domain at the N-terminus, a connecting hinge region at the C-terminus, and a kinase domain. The inactive form of Akt protein can be detected in the cytoplasm of the cell. The PH domain of akt has a strong affinity for PIP_3. (16,22) When PIP_3 binds Akt, it causes a conformational shift, exposing Akt's phosphorylation sites and activates the Akt protein, which associated in a variety of cellular functions, such as proliferation, apoptosis, cell cycle, and cytoskeletal rearrangement.(6,16) Akt can be activated by phosphorylation. The reported important phosphorylation sites were Serine (Ser)473 in Akt1, Ser474 in Akt2, and Ser472 in Akt3. In detail, Akt signaling pathway can be seen in Figure 2.

According to a recent study on oral cancer, Akt isoforms are overexpressed, hence an increase in Akt activity may cause disruption of Akt's downstream components. When Akt is active, proapoptotic proteins such as Bad and Bax are suppressed. Additionally, Akt suppresses apoptotic caspases and FOXO-1, a transcription factor involved in the regulation of proapoptotic genes such as Bim and FasL. Glycogen synthase kinase 3 (GSK-3) and FOXO activities are known to be downregulated by Akt, resulting in cyclin D1 overexpression.(16) Triggering the Akt pathway elevates cytoplasmic β-catenin as well as

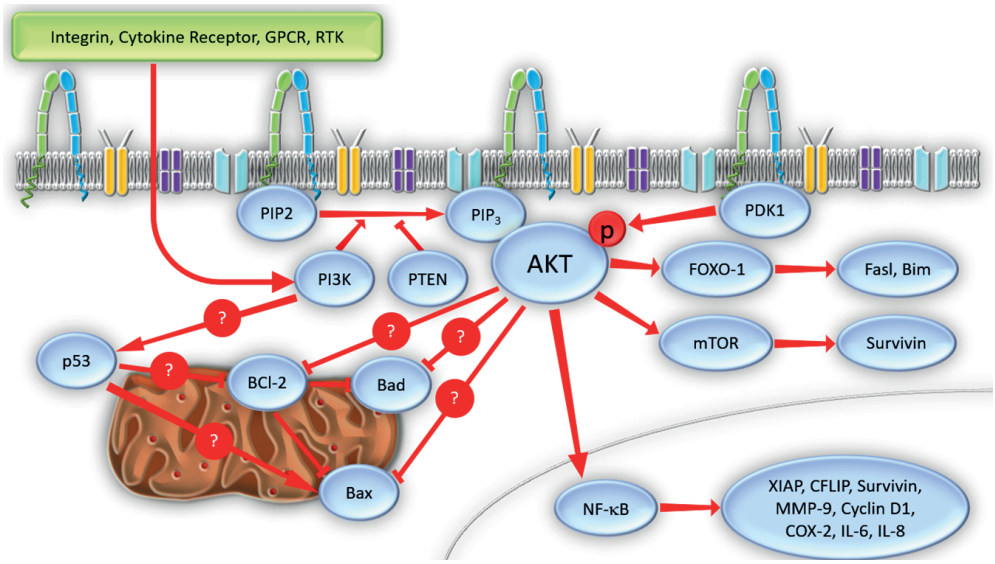

Figure 2. Schematic overview of Akt signaling pathway in ameloblastoma. Question mark: signaling pathways that have been reported in other tumors/cancers; GPCR: G Protein-coupled Receptors; RTK: Receptor Tyrosine Kinase; PTEN: Phosphatase and Tensin Homolog Dephosphorylation; PDK1: 3-phosphoinositide-dependent Protein Kinase-1; FOXO-1: Forkhead Box Protein O-1; Bim: Bcl-2 Interacting Mediator of Cell Death; p53: Tumor Protein 53; Bad: Bcl-2-Associated Death; Bax: Bcl-2 Associated X Protein; NF-κB: Nuclear Factor κB; XIAP: X-linked Inhibitor of Apoptosis Protein; cFLIP: Cellular Flice Inhibitory Protein; MMP-9: Matrix Metalloproteinase-9; COX-2: Cyclooxygenase-2 ; IL-6: Interleukin-6; IL-8: Interleukin-8.

translocate β-catenin to the nuclei, which increases c-Myc and Cyclin D1.(6) When cyclin D1 is active, it promotes the production of cyclin-dependent kinases (CDK) 4 and 6, which allows cells to leave the G1 phase and enter the S-phase, resulting in increased proliferation. Furthermore, Akt controls the cytoplasmic localization of p27, which is necessary for tumor aggressiveness and metastasis.(16)

PI3K-Akt Signaling

PI3K catalyzes the transition of membrane-bound PIP_2 to PIP_3 by phosphorylating the D3 hydroxyl group in the inositol ring of PIP_3. PIP_3 is a secondary messenger that can be deactivated by PTEN. There are four types of heterodimer PI3K: IA-PI3K, IB-PI3K, II-PI3K, and III-PI3K. Among all types, Class IA-PI3K plays the most essential role in cancer growth and development. PI3K/Akt signaling pathway modulates Inhibitor of κB (IκB)α via the induction of IκB Kinase (IKK) phosphorylation. PI3K/Akt induced activation of NF-κB by increasing the transcriptional activity of the p65 subunit.(23) PI3K/Akt activation suppresses apoptosis by increasing the transactivation potential of the p65 protein. Abnormalities in the PI3K/Akt/NF-κB signaling pathway are frequent in cancer and have a role in the development of multiple drug resistance (MDR). In chemoresistance-resistant cell lines, the PI3K/Akt/NF-κB pathway was reported to be elevated, which is associated with inhibiting apoptosis and promoting tumor growth by NF-κB.(24) It is also used as a target for resistance reversal. The PI3K/Akt pathway was implicated in the development of MDR, at least in part owing to the activation of NF-κB. PI3K/Akt/NF-κB activation in cancer cells is linked to tumorigenesis and promotes MDR-like cell proliferation and apoptosis.(25) Expressions of p53 and Bax proteins were predominantly increased in NPC HK-1 cells through modulating the PI3K/Akt/NF-κB pathway, while expression of cyclin D protein was inhibited.(26) NF-κB stimulates the transcription of cyclin D1, which later elevates cell proliferation, promotes tumor formation and medication resistance.(27)

Akt-NF-κB Signaling

Akt is also involved in the phosphorylation of IKK, which results in the activation of NF-κB.(19,28) NF-κB was initially found as a transcription factor in B-cells binding to the 11-base pair sequence enhancer element that controls the expression of immunoglobulin κ light chain.(19) It consists a group of transcription factors that promote the expressions of over 150 genes that involved in wide range of biological processes such as immune response,

inflammation, bone resorption, proliferation, angiogenesis and oncogenesis.(29-32) Furthermore, NF-κB has been shown to be a positive regulator of the expression of COX-2 in stromal cells of ameloblastoma. NF-κB stimulates both cell survival and apoptosis through a variety of regulatory mechanisms. NF-κB is known to target a large number of genes involved in inflammation (Tumor Necrosis Factor (TNF), IL-1, and chemokines), cell survival (Bcl-XL, Cellular Inhibitor of Apoptosis Proteins (cIAP), XIAP, and cFLIP), cellular immortality (telomerase), angiogenesis (Vascular Endothelial Growth Factor (VEGF), TNF, IL-8, and IL-1), proliferation (IL-6, cyclin D1, TNF, IL-1, Intercellular Adhesion Molecule (ICAM)-1, Vascular cell adhesion molecule (VCAM)-1 and Endothelial Cell Adhesion Molecule (ELAM)-1). NF-κB regulates the expression of downstream molecules and has an effect on the survival of tumor cells.(19,33)

Akt-mTOR Signaling

The Akt/mTOR pathway influences several cellular activities, including protein synthesis, autophagy, glycogen metabolism, fatty acid synthesis, nutrition absorption, nuclear protein organization.(16) The mTOR protein kinase is one of Akt main downstream targets, which is critical for cancer cell proliferation, survival, and tumorigenesis.(16,34) Tuberous sclerosis complex (TSC)-2 is phosphorylated by Akt, which suppresses its production and prevents it from activating the Ras Homolog Abundant in the Brain (RHEB). Subsequently, the RHEB-Guanosine-5'-triphosphate (GTP) activates mTOR Complex 1 (mTORC1) by binding to it. The mTORC1 complex is activated, resulting in tumor development, cell cycle progression, and decreased apoptosis.(35) The accessibility of the active sites of mTORC1 and mTORC2 are controlled by mTOR related proteins to determine their activity. Both mTOR complexes were interacted with Mammalian Lethal with SEC13 Protein 8 (mLST) and Dishevelled, Egl-10, and Pleckstrin (DEP) Domain-containing mTOR-interacting protein (DEPTOR).(16)

The Akt/mTOR pathway is critical for angiogenesis regulation in both normal and cancer tissues. Angiogenesis is the process by which cancer cells secrete VEGF, basic fibroblast growth factor (b-FGF), and interleukins such as IL-8. These cytokines bind to their receptors and activate the Akt/mTOR pathway's upstream molecules.(36) The PI3K pathway is activated, promoting angiogenesis and growth factor delivery to tumors. On the other hand, cells with aberrant mTOR signaling adapt and survive in nutrient-depleted and hypoxic conditions via alterations in the cellular response to hypoxia and nutrient absorption.(16)

Akt/mTOR pathway is involved in the epithelial-to-mesenchymal transition (EMT) regulation. Complete EMT refers to the morphologically obvious transition from the epithelial to the mesenchymal state. Epithelial cells lose their epithelial characteristics and acquire mesenchymal characteristics, including loss of apical-basal polarity, loss of cell-cell contact, and disassembly of the actin cytoskeleton. On the other hand, incomplete or partial EMT refers to the presence of intermediate hybrid epithelial and mesenchymal phenotypes.(37) PTEN has been implicated in the regulation of the EMT in both development and cancer.(38) Loss of PTEN enables the over-activated PI3K/Akt pathway to induce its downstream, resulting in EMT, which further promotes invasion. This pathway enables cell proliferation to be accelerated, apoptosis to be inhibited, and the cell cycle to be deregulated.(39,40)

Additionally, the Akt/mTOR axis regulates other critical cell activities. For example, it modulates the dysregulation of GSK-3, a protein involved in glycogen synthesis. Moreover, this pathway's components regulate the expression of ATP citrate lyase (ACLY), a well-characterized fatty acid synthase enzyme. Furthermore, this pathway regulates glucose transport proteins such as phosphatidylinositol-4-phosphate 5-kinase (PIP5K) and AS160, glycolysis-related proteins such as hexokinase and 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 2 (PFKFB2), and nuclear protein organization via Lamin A. As a result, the Akt/mTOR pathway is essential in carcinogenesis and regulates a variety of cellular functions.(16) Human ameloblastoma tissues expressed significantly more phosphorylated Akt (p-Akt) and p-mTOR than normal oral mucosa, demonstrating the Akt/mTOR axis' clinical relevance.(7,16)

Akt-Bcl-2 Family Signaling

The Akt/Bcl-2 pathway is primarily involved in apoptosis inhibition but may also be involved in proliferation of cells, tumor development, aggressive clinical behavior, and oncogenesis of the odontogenic epithelium. Bcl-2 was detected in the the tumor cell's outer layer, suggesting that it has proliferative activity and suppresses cell death, and it may reflect growth of ameloblastoma and protects tumor cells by inhibiting apoptosis.(41) Bcl-2 inhibits Bax's proapoptotic function and promoting cell proliferation, as

evidenced in each type of ameloblastoma's biological tumor behavior. The Bcl-2 protein significantly inhibits apoptosis in ameloblastoma tumor cells. The high level of Bcl-2 expression in ameloblastoma cells, specifically in basal cells with inverse polarization, may have an effect on the ameloblastoma's behavior.(42)

Bax is an apoptotic promoter with a similar structure to Bcl-2 and the ability to form homodimers and heterodimers with Bcl-2. The interaction between Bcl-2 and Bax determines the survival or death of cells. When Bax activity exceeds Bcl-2 activity, Bax is suppressed and the Bax apoptotic pathway is activated.(43) Bcl-2 inhibition induces apoptosis in ameloblastoma cells and inhibited tumor formation in immunodeficient mice, demonstrating that Bcl-2 influences ameloblastoma tumorigenesis via apoptosis regulation.(44) Additionally, p53 influences the expression of Bcl-2 and Bax, with a positive correlation with Bcl-2 and a negative correlation with Bax. Thus, there is a significant correlation between p53-overexpressing odontogenic lesions and increased anti-apoptotic factor (Bcl-2) expression, as well as decreased pro-apoptotic factor expression (Bax). This might be compounded by the fact that, under normal circumstances, p53 directly triggers apoptosis. Antiapoptotic factors, on the other hand, are overexpressed in pathological circumstances such as neoplasms, encouraging cell survival and evading cell cycle regulation systems.(45)

Akt-Survivin Signaling

Survivin, an IAP family member, functions in malignant tumors by inhibiting apoptosis and promoting cell proliferation. As a result of alternatively spliced transcripts, Survivin exists in five distinct isoforms: Survivin, Survivin-2B, Survivin-Ex3, Survivin-3B, and Survivin-2 are all members of the Survivin family.(46) Survivin is expressed in the nuclei of tumor cells during the G2/M phase of the cell cycle, indicating a poor prognosis in a variety of malignant tumors.(47) According to recent research in oral cancer, the Akt1 and Akt2 isoforms promote cell survival and inhibit cell cycle arrest at the G2/M phase, in addition to releasing expression of proteins engaged in cell proliferation and survival, such as COX-2, cyclin D1, and Survivin, as well as the anti-apoptotic protein Bcl-2.(16) Survivin protein is located in the nucleus of ameloblastic cells, where nuclear Survivin immunoexpression promotes cell proliferation, while cytoplasmic Survivin expression is related in apoptosis regulation mechanisms.(42)

Potential Target on Akt Signaling of Ameloblastoma

According to some reports, the PI3K pathway is involved in the development of ameloblastoma. This pathway is also involved in cancer cell proliferation and survival, which explains why PI3K pathway inhibitors have been rapidly developed as cancer therapeutics.(34) PI3K signaling is regarded to be a major therapeutic target for cancer therapy, since it is highly associated with human tumor growth, increased tumor microvessel density, and increased chemotaxis and invasive capability of cancer cells.(48) These may be beneficial in treating ameloblastoma.(34)

As previously stated, there are four types of PI3K, with the IA-PI3K being the most likely to be involved in cancer. There are three distinct PI3K isoforms, p110α, p110β, and p110γ, each with distinct but overlapping roles in cancer. There have been developed PI3K inhibitors that inhibit all isoforms or isoform-specific inhibitors. The advantage of isoform-specific inhibitors is their ability to inhibit specific targets with few adverse effects. By inhibiting PI3K signaling, we can avoid the toxicities associated with direct inhibition of mTORC1 and mTORC2, thereby making them more tolerable. Inhibiting PI3K-Akt signaling in tumors has been demonstrated to be effective using inhibitors such as p110-specific inhibitors. Additionally, there is mounting evidence that p110 inhibitors may be critical in the treatment of certain PTEN-deficient cancers, including ameloblastoma.(34)

Chemical Compounds

Several chemical compounds have been developed to target Akt (Table 1). Wortmannin and LY294002 are both effective PI3K inhibitors. Both are often used to inhibit cell growth through the PI3K/Akt signaling pathway. Wortmannin is a reagent that has been frequently utilized in cell biology to hinder DNA repair, receptor-mediated endocytosis, and cell proliferation.(49,50) LY294002 is a synthetic compound derived from the flavonoid quercetin that was developed as a PI3K inhibitor. LY294002 acts by competing with ATP for binding to the PI3K active site.(50)

LY294002 inhibits PI3K specifically while having no effect on other protein kinases such as AMP-dependent protein kinase or c-Src. Apart from PI3K, it has been shown that LY294002 inhibits a variety of other signaling components, including NF-κB, heat shock proteins (HSP)

Table 1. Various means of potential target on Akt signaling of ameloblastoma.

	Therapy	Means of Inhibition
Compounds	MK-2206	Akt
	Perifosine	Akt
	CAL-101 (Idelalisib)	PI3K
	IPI-145 (Duvelisib)	PI3K
	Wortmannin	PI3K
	LY294002	PI3K
miRNA	miR-181d	Akt
	miR-29	Akt 2 & Akt 3
	miR-126	PI3K
CRISPR/Cas9		Akt & PI3K

27 and 72, Akt, and Survivin.(50) SF1126 is a PI3K and mTOR inhibitor. It consists of a LY294002-tetrapeptide conjugation linked to an Arg-Gly-Asp-Ser (RGDS) tetrapeptide. This prodrug's permeability is increased by its ability to bind to particular integrins within the tumor. SF1126, a PI3K inhibitor, inhibits both PI3K and the RAS-MAP kinase pathways.(16,51) In a PTEN-loss scheme, Akt signaling pathway may be inhibited with a PI3K inhibitor such as LY294002, wortmannin, or another inhibitor.(40)

Perifosine is an oral Akt inhibitor that is undergoing Phase III clinical studies at the moment. Perifosine (KRX-0401, D-21266) is a laboratory-synthesized heterocyclic alkylphospholipids (APL) analog that affects signal transduction pathways at the cell membrane. Perifosine inhibits Akt phosphorylation at the threonine (T308) and serine (S310) sites (S473). Perifosine's antitumor effect was also beneficial when combined with radiation, implying that perifosine has a favorable profile in combination therapies.(52) MK-2206, another Akt inhibitor, is currently being studied in tumors with PIK3CA mutations or Akt mutations, as well as PTEN loss/mutation. MK-2206 was shown in preclinical studies to inhibit Akt signaling *in vitro* and tumor growth *in vivo* in a dose-dependent manner. Numerous clinical studies have been conducted using MK-2206, which has shown modest effectiveness in renal cell carcinoma and moderate activity in lymphoma. MK-2206 in combination with trastuzumab and MK-2206 in combination with ridaforolimus have demonstrated to be more efficacious.(53)

Clinical trials on drugs targeting PI3K have been conducted, and only a few drugs have been approved by the FDA for use in humans, including IPI-145 (Duvelisib), an oral dual inhibitor of PI3K and PI3K, and CAL-101 (Idelalisib), an oral specific inhibitor of PI3K.(48) However, no research has been conducted to determine whether these medications are effective in the treatment of ameloblastoma.

micro RNA

Micro RNA (miRNA/MiR) has been linked to tumor proliferation and metastasis and is associated with tumor development and progression.(54) miR-181d, one of the miRNAs, has been shown to act as a tumor suppressor, inhibits proliferation, migration, and cell cycle arrest in esophageal squamous cell carcinoma and glioma.(55) miR-181d functioned as a tumor suppressor by inhibiting HGC-27 gastric cancer cells proliferation via PI3K/Akt pathway CYLD/PI3K/Akt pathway and invasion-mediated EMT via targeting CYLD.(56)

miR-29a, mir-29b, and mir-29c are all members of the miR-29 family. With identical seed sequences and similar expression patterns and functions, miR-29 has been shown to play a critical role in a variety of pathophysiological processes.(57,58) One research addressing the function of miR-29 in cancer metabolism revealed that miR-29b was downregulated in ovarian cancer, inhibiting glycolysis and glucose metabolism in cancer cells by direct targeting of Akt2 and Akt3.(59) Along with facilitating tumor suppression through glucose metabolism regulation, miR-29 has been shown to act as a tumor suppressor in glioblastoma multiforme (GBM) via an autoregulatory loop involving Sterol Regulatory Element-binding Protein 1 (SREBP-1), a transcription factor that regulates genes involved in sterol biosynthesis, glucose and lipid metabolism, and has been shown to promote tumorigenesis.(58,60)

Another micro RNA, miR-126, was found to inhibit the PI3K/Akt pathway in SLK cells in Kaposi sarcoma, affecting SLK cell proliferation by inhibiting the cell cycle, inducing cell apoptosis, and decreasing cell invasiveness. Additionally, miR-126 has been shown to inhibit PI3K/Akt in a variety of cancers and diseases, including leukemia and renal diseases.(61,62) Although these findings regarding several miRNAs are extremely promising for future treatments of ameloblastoma, further validation in ameloblastoma cases is required.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

CRISPR systems generate a huge potential for gene mutation targeting. In recent years, disease-causing mutations

targeted by CRISPR systems have been a primary focus of research; this is especially true for diseases for which there is currently no cure, such as cancer.(63) CRISPR associated protein 9 (Cas9) is the most used CRISPR tool, consisting of two components: single guide RNA (sgRNA) and the Cas9 subtype of Cas proteins. CRISPR sequences serve as a guide for the Cas9 enzyme, resulting in the formation of a complex capable of identifying and cutting complementary sequences. This allows for the insertion of the desired gene into the cleaved and removed site.(64) Studies using CRISPR has been shown to regulate downstream molecules such as PI3K, ERK, Akt, Stat3, and c-myc, and some studies have been conducted using CRISPR to target PI3K in pancreatic cancer.(65) Although CRISPR has been shown a great potential and used to edit genes associated with a variety of diseases, its clinical application in cancer is still in its infancy, and there are currently no studies using CRISPR to treat ameloblastoma cases.

Conclusion

While surgery remains the gold standard for ameloblastoma treatment, there is considerable potential for inhibiting ameloblastoma in a variety of ways. Thus, it is necessary to understand the underlying signaling in ameloblastoma in order to induce inhibition. Additional research into potential inhibitors of Akt signaling in ameloblastoma will result in improved management of the disease in the future.

Authors Contribution

Manuscript was proposed and supervised by FS, drafted and designed by SH and LAP. All authors made manuscript revisions and finalized the last version of the manuscript.

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