

## REVIEW ARTICLE

## Targeting Ameloblastoma into Apoptosis

Ferry Sandra<sup>1,2,\*</sup><sup>1</sup>Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Trisakti University, Jl. Kyai Tapa No.260, Jakarta, Indonesia<sup>2</sup>BioCORE Laboratory, Faculty of Dentistry, Trisakti University, Jl. Kyai Tapa No.260, Jakarta, Indonesia

\*Corresponding author. E-mail: ferrysandra@gmail.com

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## Abstract

**BACKGROUND:** Generally ameloblastoma is a locally aggressive, slow growing, non-metastatic epithelial odontogenic benign tumor. However, rarely some ameloblastoma can metastasize in spite of a benign histologic appearance. Targeting ameloblastoma by inducing it into apoptosis could be a beneficial strategy, since many ameloblastoma cases were reported recurrent after surgical therapy.

**CONTENT:** To investigate ameloblastoma in cellular aspect, cytological pattern of ameloblastoma was divided into outer layer/peripheral and inner layer/central cells. Tumor necrosis factor (TNF)- $\alpha$ , Fas ligand (FasL), TNF receptor (TNFR)1/death receptor (DR)1, TNFR2/DR2, DR4, DR5 and Fas were highly expressed in central than peripheral cells. Despite inducing apoptosis, TNF- $\alpha$  can induce PI3K leading to Akt and p44/42 mitogen-activated protein kinases (MAPK) activation in AM-1 cells, which later induce cell survival and proliferation. Therefore apoptotic induction in ameloblastoma should be suggested in higher TNF- $\alpha$  concentration. Expression of FasL and Fas are closely associated with squamous metaplasia and

granular transformation of the tumor cells, suggesting that apoptosis induced by FasL may play a role in the terminally differentiated or degenerative ameloblastoma cells. TNF-related apoptosis-inducing ligand (TRAIL) has emerged as an apoptotic inducing anticancer agent in tumor cells specifically. TRAIL induced activation of caspases, lowering mitochondrial membrane potential, high number of apoptotic cells in ameloblastoma cells. Therefore, TRAIL could be a potential agent for targeting ameloblastoma, although further study should be explored.

**SUMMARY:** Targeting ameloblastoma by inducing it into apoptosis could be achieved effectively, although some criteria should be considered. Therefore understanding the underlying apoptosis signaling pathways are necessary for inducing ameloblastoma into apoptosis. Investigations on other apoptosis-related molecules, potential apoptosis-inducing natural products, and novel approach in reprogramming, are important in the future for a better management of ameloblastoma.

**KEYWORDS:** ameloblastoma, apoptosis, TNF, Fas, TRAIL, Akt, MAPK, caspase

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## Introduction

Generally ameloblastoma is a locally aggressive, slow growing, non-metastatic epithelial odontogenic benign tumor.(1,2) However, rarely some ameloblastoma can metastasize in spite of a benign histologic appearance.(3,4)

Ameloblastoma has several histopathologic patterns, including follicular, plexiform, desmoplastic, basal cell, acanthomatous, desmoplastic and granular cell. While clinically ameloblastoma is classified into solid, cystic and peripheral.(4)

To investigate ameloblastoma in cellular aspect, cytological pattern of ameloblastoma was divided into outer

layer/peripheral and inner layer/central cells.(3,5-13) The peripheral cells were further classified into basal, columnar and cuboidal cell types.(5,7,8,10) Immunohistochemical studies have shown that B cell lymphoma (Bcl)-2 (3,5,7), Bcl-x (3,6) proliferating cell nuclear antigen (PCNA) (5), Ki-67 (5), murine double minute 2 (MDM2) (8,9), Midkine (10) and protein (p)65 nuclear factor (NF)  $\kappa$ B (13) were highly expressed in peripheral than in central cells. Meanwhile, tumor necrosis factor (TNF)- $\alpha$  (11), Fas ligand (FasL) (14), TNF receptor (TNFR)1/death receptor (DR)1 (11), TNFR2/DR2 (11), DR4 (12), DR5 (12) and Fas (14) were highly expressed in central than peripheral cells.

In accordance with the expressions of apoptotic ligand and receptor in central cells, expressions of p53 (8,15), phosphatase and tensin homolog (PTEN) (16), phosphorylated-PTEN (16), phosphorylated-Jun N-terminal kinase (JNK) (17), Bcl-2 associated X protein (Bax) (3,6), Bcl-2 homologous antagonist killer (Bak) (3,5,7), caspase 9 (18), apoptotic protease activating factor-1 (APAF-1) (18), caspase 3 (19), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (14,20) were observed in central cells as well. Hence, some reports suggested that central cells have higher apoptotic activity than peripheral cells.(3,6,14,20) Targeting ameloblastoma by inducing it into apoptosis could be a beneficial strategy, since many ameloblastoma cases were reported recurrent after surgical therapy.(1)

## Apoptosis-inducing Ligands

### TNF- $\alpha$

TNF- $\alpha$  is mainly produced by macrophages and monocytes. TNF- $\alpha$  plays important roles in cell proliferation, apoptosis, survival and differentiation.(11,21) TNF- $\alpha$  depends on its concentration might induce different mechanisms: a low concentration of TNF- $\alpha$  mainly induces phosphatidylinositol-3-OH kinase (PI3K)/Akt/inhibitor of  $\kappa$ B (I $\kappa$ B) kinase (IKK)/I $\kappa$ B survival signaling pathway.(21) Meanwhile, a high concentration of TNF- $\alpha$  mainly induces apoptosis.(21) Inhibition of low concentration of TNF- $\alpha$ -induced survival signaling pathway with wortmannin, LY294002, kinase negative (KN)-Akt, KN-IKK- $\alpha$ , KN-IKK- $\beta$  or undegradable I $\kappa$ B (undeg-I $\kappa$ B), will turn low concentration TNF- $\alpha$  to induce apoptosis (Figure 1).(21) KN DNA constructs including KN-Akt, KN-IKK- $\alpha$  and KN-IKK- $\beta$ , were generated as mutated competitive agents having defected kinase function.(21) Meanwhile, undeg-I $\kappa$ B was generated as competitive agent with mutated

phosphorylation site. Hence, I $\kappa$ B will not be labeled for degradation.(21)

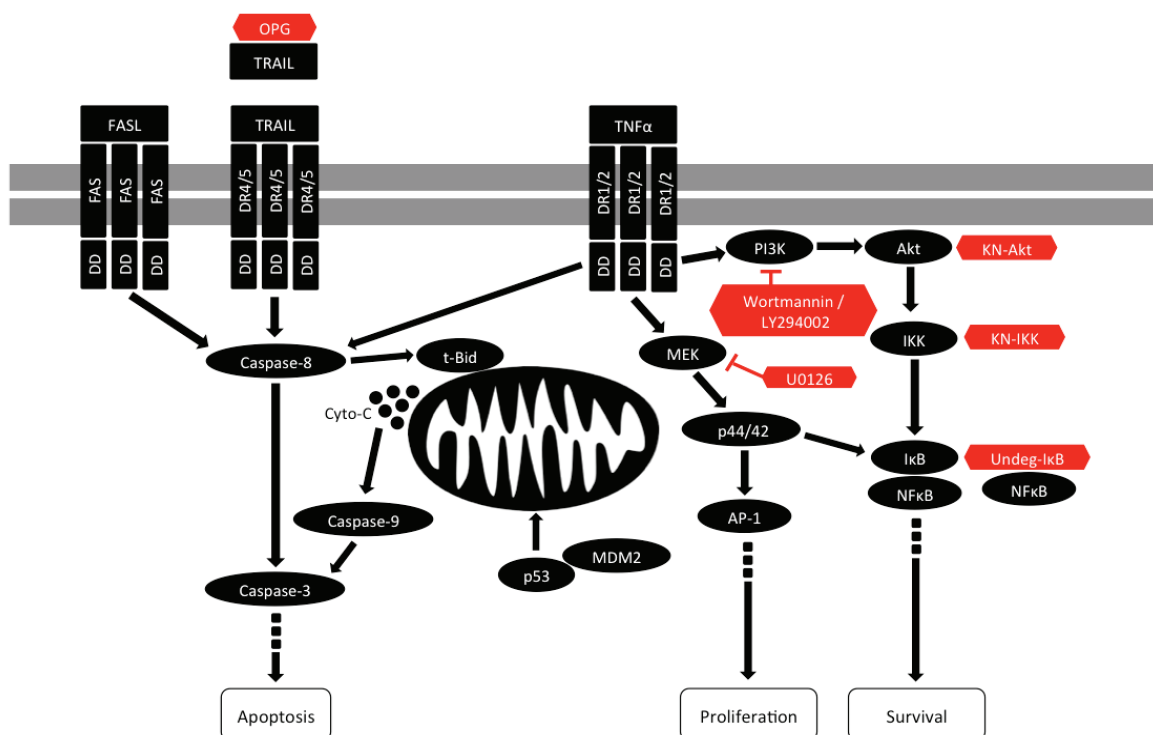
TNF- $\alpha$ , TNF Receptor (TNFR)1 and TNFR2, are expressed in AM-1 cells and ameloblastoma.(11) AM-1 cells, a cell line of ameloblastoma, have been widely used as ameloblastoma cell model.(8-13,22) Despite inducing apoptosis, 1-100 ng/mL TNF- $\alpha$  can induce PI3K leading to Akt and p44/42 mitogen-activated protein kinases (MAPK) activation in AM-1 cells, which later induce cell survival and proliferation.(11) Therefore apoptotic induction in ameloblastoma should be suggested in higher TNF- $\alpha$  concentration.

### FasL

FasL, a cell-surface molecule belonging to TNF family, binds to its receptor Fas, a member of the TNFR family. The binding triggers a series of intracellular signal transduction leading to the activation of caspases. Activated-caspases execute the apoptotic process by cleaving various substrates.(14) Expression of FasL and Fas was detected in the majority of ameloblastomas. A strong staining was observed in the central area of tumor islands. Close association was reported between the expression of FasL-Fas and squamous metaplasia / granular transformation of the tumor cells. This suggest that apoptosis induced by FasL may play a role in the terminally differentiated or degenerative ameloblastoma cells.(15) Hence targeting Fas for ameloblastoma apoptotic induction could be more suitable for more differentiated types of ameloblastoma, such as plexiform or follicular, but not basal cell type.

### TNF-related Apoptosis-inducing Ligand (TRAIL)

TRAIL, a potent ligand in inducing apoptosis, has emerged as an apoptotic inducing anticancer agent in tumor cells specifically. Due to its activity on ameloblastoma, an investigation has been reported.(12) Expressions of DR4 and DR5, TRAIL's receptors, were detected in all ameloblastoma types and AM-1 cells. By applying TRAIL in AM-1 cells for 24 hours, cleavages of caspase-8, -9 and -3 were formed (Figure 1).(12) Induction of mitochondrial apoptotic pathway was confirmed, marked by lowering mitochondrial membrane potential. High number of apoptotic cells were noticed in AM-1 cells upon treatment of TRAIL for 24 hours.(12) Hence TRAIL could be a potential agent for targeting ameloblastoma, although further study should be explored to confirm this evidence. In addition, Osteoprotegerin (OPG), an inhibitor should be avoided when the TRAIL was suggested as the targeting agent.



**Figure 1. Apoptosis signaling pathway in ameloblastoma.** Several major pathways have been clarified and shown to be effective to induce apoptosis in ameloblastoma. AP-1: Activator protein-1; DD: Death Domain; DR: Death Receptor; Cyto-C: Cytochrome-C; t-Bid: truncated B cell lymphoma (Bcl)-2 homology (BH)3 interacting-domain death agonist.

OPG has been reported as a useful receptor to inhibit Receptor Activator of NFκB Ligand (RANKL) in inducing osteoclastogenesis.(22) Expression of OPG was observed in AM-1 cells and ameloblastoma. Since OPG was shown to bind with TRAIL as well, in an apoptotic assay with AM-1 cells, OPG could suppress TRAIL's capacity in inducing apoptosis.(23) Therefore, osteoclastogenesis suppression using OPG on ameloblastoma-mediating-bone destruction should be considered, since the OPG can also suppress TRAIL's function in inducing apoptosis in ameloblastoma.

## Apoptosis-related Secondary Messengers

### p53/MDM2

The p53 protein, known as a potent cell growth inhibitor, is responsible for arresting the cell cycle at several distinct points, as well as activating the apoptotic signal transduction in some circumstances.(8) Cellular stresses can activate p53, including DNA damage and hypoxia. Investigation in ameloblastoma has shown that most of wild type p53 population was detected in ameloblastoma but the p53 labeling indices were not correlated neither with WHO classification nor the cytological pattern of the outer layer cells of ameloblastoma.(8)

MDM2 can modulate tumor suppressor activity of p53 and mediate degradation of p53. The degradation of p53 can be triggered by shuttling p53 from nucleus to cytoplasm. MDM2 was detected and correlated with cytological pattern of the outer layer cells of ameloblastoma. Hence, p53 in ameloblastoma might have a function in the cell cycle, however p53 suppressor activity might be suppressed by MDM2.(8,9) Therefore, a strategy in inhibiting MDM2 could be suggested and explored further to regain p53's role as cell growth inhibitor or apoptosis activator.

### PI3K/Akt/Mammalian-target of Rapamycin (mTOR)

In carcinogenesis, loss of PTEN allows the over-activated PI3K/Akt pathway in inducing its downstream. This pathway allows acceleration of proliferation, inhibition of apoptosis and deregulation of cell cycle. An allelic loss of PTEN was reported to be occurred in ameloblastomas.(16) The aberrant expression of PTEN in ameloblastomas was reported to be correlated with the inherent aggressiveness of ameloblastoma.(16)

In the study using AM-1 cells, as mentioned earlier, the phosphorylation of Akt (Ser473) could be induced by TNF-α. Akt phosphorylation is usually associated with cell survival. Therefore, in ameloblastoma, Akt phosphorylation should be inhibited and the attempt can be achieved by

pretreatment of LY294002, a PI3K inhibitor.(11) Besides TNF- $\alpha$ , a protein expressed during tooth development in the epithelium of the odontogenic apparatus or its remnant tissues called Midkine, could induce phosphorylation of Akt (Ser473) and Thr308.(10) These results support some explanation regarding how remnant tissues can potentially develop into ameloblastoma. Targeting Akt signaling pathway in a PTEN-loss scheme can be suggested by using PI3K inhibitor, such as LY294002, wortmannin, or other equal inhibitors (Figure 1).

#### p42/44 MAPK

Some studies have shown the effects of MAPK inducers in ameloblastomas. Besides playing a critical role in cell proliferation, MAPK plays a role in cell survival as well. TNF $\alpha$  induced phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) (Thr202/Tyr204) in AM-1 cells.(11) The phosphorylation can later be inhibited by pretreatment of U0126, mitogen-activated extracellular-regulated kinase (MEK) 1/2 inhibitor.(24) Therefore apoptosis induction in ameloblastoma can be achieved, besides applying higher TNF- $\alpha$  concentration, a MEK 1/2 inhibitor, such as U0126 or other equal inhibitors, could be suggested (Figure 1).

#### Future Research Perspective

Major targets have been reported to induce ameloblastoma into apoptosis as described above. Other second messengers, such as Bcl-2 family (3,6), Survivin (15,25), APAF-1 (18), X chromosome-linked inhibitor of apoptosis protein (XIAP) have been reported, but there was not any functional study related to these molecules.(25) Therefore those molecules should be further investigated, so that a more complete strategy in inducing ameloblastoma into apoptosis could be obtained.

Due to apoptotic induction, as a substitute for chemicals, natural products have been proposed to avoid possible side effects caused. There are studies of herbal extracts in targeting cancer cells, such as caffeic acid (26), artocarpin (27), *Curcuma mangga* (28), *Kleinhovia hospita* L. (29), Kayu Secang (30), *Piper betle* L. (28), *Catharanthus roseus* [L] G.Don (28), *Dendrophthoe petandra* L. (28,31), *Nephelium lappaceum* L. (28), *Curcuma mangga* Val. (28), *Brucea javanica* (31,32), *Artocarpus altilis* (33,34), *Piper crocatum* Ruiz & Pav (35) and prenylated flavonoids (36). These potential extracts should be further investigated on ameloblastoma.

Research in cancer cells genetic modification has been started and shown as a potential approach in managing

cancer. Cell reprogramming for turning differentiated cells into stem cells has become a trending technology. (37) In cancer research, reversing the breast cancer stem cell into breast somatic stem cell has been reported.(38) The reprogramming/reversing could be conducted with a single gene or series of genes modifications. Or simply by changing the microenvironment, as being conducted in stem cell research for differentiating stem cell.(39) Meanwhile, in cancer research, modification on the microenvironment has also been reported.(40) Therefore, this potential strategy should be further investigated on ameloblastoma as well.

#### Conclusion

Targeting ameloblastoma by inducing it into apoptosis could be achieved effectively, although some criteria should be considered. Not only apoptosis-inducing ligand, but second messengers also play crucial role in apoptotic induction. Therefore understanding the underlying apoptosis signaling pathways are necessary for inducing ameloblastoma into apoptosis. Investigations on other apoptosis-related molecules, potential apoptosis-inducing natural products, and novel approach in reprogramming, are important in the future for a better management of ameloblastoma.

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