REVIEW ARTICLE

Targeting Ameloblatoma into Apoptosis

Ferry Sandra[1,2,*]

[1]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Trisakti University, Jl. Kyai Tapa No.260, Jakarta, Indonesia
[2]BioCORE Laboratory, Faculty of Dentistry, Trisakti University, Jl. Kyai Tapa No.260, Jakarta, Indonesia

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

Received date: Aug 11, 2017; Revised date: Jan 8, 2018; Accepted date: Jan 9, 2018

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)-α, 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-α 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-α 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 ameloblasotma 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

Indones Biomed J. 2018; 10(1): 35-9

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) κB (13) were highly expressed in peripheral than in central cells. Meanwhile, tumor necrosis factor (TNF)-α (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-α

TNF-α is mainly produced by macrophages and monocytes. TNF-α plays important roles in cell proliferation, apoptosis, survival and differentiation.(11,21) TNF-α depends on its concentration might induce different mechanisms: a low concentration of TNF-α mainly induces phosphatidylinositol-3-OH kinase (PI3K)/Akt/inhibitor of κB (IκB) kinase (IKK)/IκB survival signaling pathway.(21) Meanwhile, a high concentration of TNF-α mainly induces apoptosis.(21) Inhibition of low concentration of TNF-α-induced survival signaling pathway with wortmannin, LY294002, kinase negative (KN)-Akt, KN-IKK-α, KN-IKK-β or undegradable IκB (undeg-IκB), will turn low concentration TNF-α to induce apoptosis (Figure 1).(21) KN DNA constructs including KN-Akt, KN-IKK-α and KN-IKK-β, were generated as mutated competitive agents having defected kinase function.(21) Meanwhile, undeg-IκB was generated as competitive agent with mutated phosphorylation site. Hence, IκB will not be labeled for degradation.(21)

TNF-α, 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-α 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-α 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.

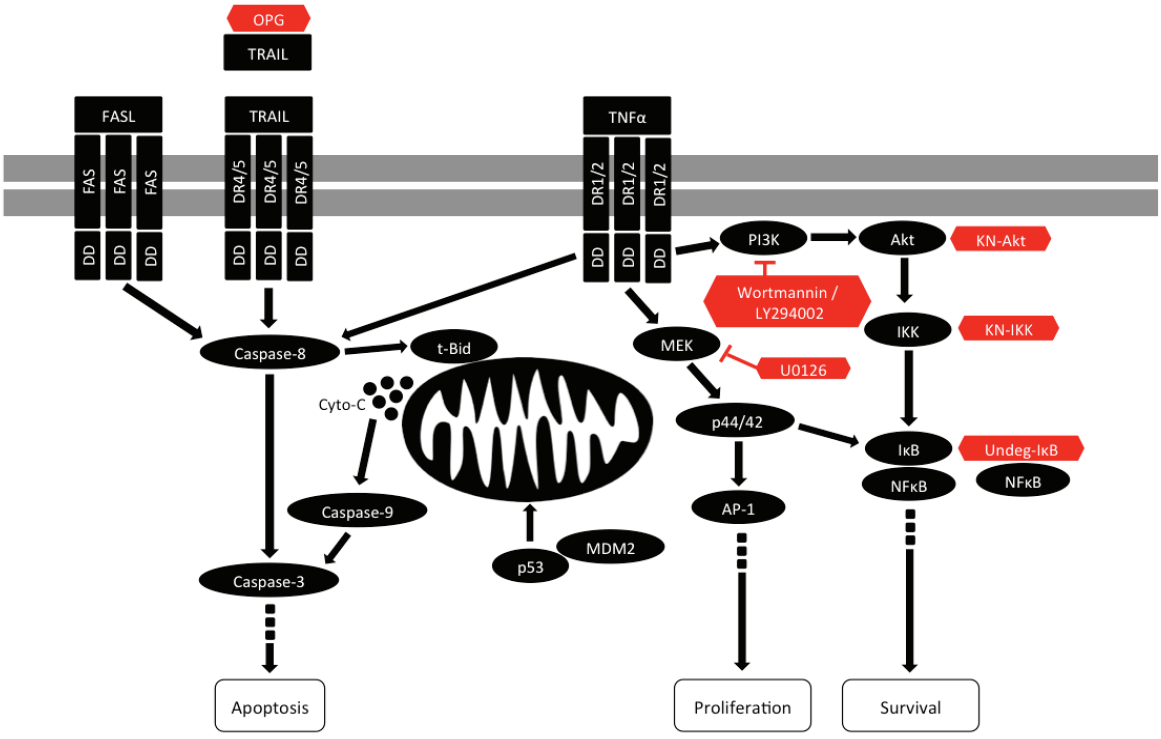

Figure 1. Apoptosis signaling pathway in ameloblastoma. Several major pathways have been clarified and shown to be effective to induce apoptosis in ameloblastoma. AP-1: Activator protein-1; DD: Death Domain; DR: Death Receptor; Cyto-C: Cytochrome-C; t-Bid: truncated B cell lymphoma (Bcl)-2 homology (BH)3 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 ameloblatoma, Akt phosphorylation should be inhibited and the attempt can be achieved by

pretreatment of LY294002, a PI3K inhibitor.(11) Besides TNF-α, 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. TNFα 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-α 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), *Dendrophtoe 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 ameloblasotoma.

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 became 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 ameloblasotma 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.

References

1. Nakamura N, Mitsuyasu T, Higuchi Y, Sandra F, Ohishi M. Growth characteristics of ameloblastoma involving the inferior alveolar nerve: a clinical and histopathologic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001; 91: 557-62.
2. Sandra F. The proliferative and apoptotic behaviors of ameloblastoma [Dissertation]. Fukuoka: Kyushu University; 2001.
3. Sandra F, Nakamura N, Takeuchi H, Misuyasu T, Shiratsuchi Y, Ohishi M. The Analysis of Apoptosis in Ameloblastoma: Evaluation of Bcl-2, Bcl-X, Bax, Bak. J Dent Indones. 2000; 7: 483-5
4. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization Classification of Tumors. Pathology and Genetics of Head and Neck Tumors. Lyon: IARC Press; 2005.
5. Sandra F, Mitsuyasu T, Nakamura N, Shiratsuchi Y, Ohishi M. Immunohistochemical evaluation of PCNA and Ki-67 in ameloblastoma. Oral Oncol. 2001; 37: 193-8 .
6. Sandra F, Nakamura N, Mitsuyasu T, Shiratsuchi Y, Ohishi M. Two relatively distinct patterns of ameloblastoma: an anti-apoptotic proliferating site in the outer layer (periphery) and a pro-apoptotic differentiating site in the inner layer (centre). Histopathology. 2001; 39: 93-8.
7. Nakamura N, Higuchi Y, Mitsuyasu T, Sandra F, Ohishi M. Comparison of long-term results between different approaches to ameloblastoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002; 93: 13-20.
8. Sandra F, Nakamura N, Kanematsu T, Hirata M, Ohishi M. The role of MDM2 in the proliferative activity of ameloblastoma. Oral Oncol. 2002; 38: 153-7.

9. Sandra F. In reply to "Comment on: F. Sandra *et al*., The role of MDM2 in the proliferative activity of ameloblastoma", Oral Oncology 2002; 38: 153-7. Oral Oncol. 2003; 39: 745.
10. Sandra F, Harada H, Nakamura N, Ohishi M. Midkine induced growth of ameloblastoma through MAPK and Akt pathways. Oral Oncol. 2004; 40: 274-80.
11. Hendarmin L, Sandra F, Nakao Y, Ohishi M, Nakamura N. TNFalpha played a role in induction of Akt and MAPK signals in ameloblastoma. Oral Oncol. 2005; 41: 375-82.
12. Sandra F, Hendarmin L, Nakao Y, Nakamura N, Nakamura S. TRAIL cleaves caspase-8, -9 and -3 of AM-1 cells: a possible pathway for TRAIL to induce apoptosis in ameloblastoma. Tumour Biol. 2005; 26: 258-64.
13. Hendarmin L, Kawano S, Yoshiga D, Sandra F, Mitsuyasu T, Nakao Y, *et al.* An anti-apoptotic role of NF-κB in TNFα-induced apoptosis in an ameloblastoma cell line. Oral Sci Int. 2008; 5: 96-103.
14. Luo HY, Yu SF, Li TJ. Differential expression of apoptosis-related proteins in various cellular components of ameloblastomas. Int J Oral Maxillofac Surg. 2006; 35: 750-5.
15. Shaikh Z, Niranjan KC. Cell cycle aberration in ameloblastoma and adenomatoid odontogenic tumor: As evidenced by the expression of p53 and survivin. Indian J Dent Res. 2015; 26: 565-70.
16. Scheper MA, Chaisuparat R, Nikitakis NG, Sauk JJ. Expression and alterations of the PTEN / AKT / mTOR pathway in ameloblastomas. Oral Dis. 2008; 14: 561-8.
17. Kumamoto H, Ooya K. Immunohistochemical detection of phosphorylated JNK, p38 MAPK, and ERK5 in ameloblastic tumors. J Oral Pathol Med. 2007; 36: 543-9.
18. Kumamoto H, Ooya K. Detection of mitochondria-mediated apoptosis signaling molecules in ameloblastomas. J Oral Pathol Med. 2005; 34: 565-72.
19. Khalifa GA, Shokier HM, Abo-Hager EA. Evaluation of neoplastic nature of keratocystic odontogenic tumor versus ameloblastoma. J Egypt Natl Canc Inst. 2010; 22: 61-72.
20. Amaral FR, Mateus GC, Bonisson LA, de Andrade BA, Mesquita RA, Horta MC, *et al.* Cell proliferation and apoptosis in ameloblastomas and keratocystic odontogenic tumors. Braz Dent J. 2012; 23: 91-6.
21. Sandra F, Matsuki NA, Takeuchi H, Ikebe T, Kanematsu T, Ohishi M, *et al.* TNF inhibited the apoptosis by activation of Akt serine/threonine kinase in the human head and neck squamous cell carcinoma. Cell Signal. 2002; 14: 771-8.
22. Sandra F, Hendarmin L, Kukita T, Nakao Y, Nakamura N, Nakamura S. Ameloblastoma induces osteoclastogenesis: a possible role of ameloblastoma in expanding in the bone. Oral Oncol. 2005; 41: 637-44.
23. Sandra F, Hendarmin L, Nakamura S. Osteoprotegerin (OPG) binds with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): Suppression of TRAIL-induced apoptosis in ameloblastomas. Oral Oncol. 2006; 42: 415-20
24. Sandra F, Hendarmin L, Nakao Y, Nakamura N, Nakamura S. Inhibition of Akt and MAPK pathways elevated potential of TNFalpha in inducing apoptosis in ameloblastoma. Oral Oncol. 2006; 42: 39-45.
25. Kumamoto H, Ooya K. Expression of survivin and X chromosome-linked inhibitor of apoptosis protein in ameloblastomas. Virchows Arch. 2004; 444: 164-70.
26. Sandra F, Sidharta MA. Caffeic Acid Induced Apoptosis in MG63 Osteosarcoma Cells Through Activation of Caspases. Mol Cell Biomed Sci. 2017; 1: 28–33.
27. Arung ET, Wicaksono BD, Handoko YA, Kusuma IW, Shimizu K, Yulia D, *et al.* Cytotoxic effect of artocarpin on T47D cells. J Nat Med. 2010; 64: 423-9.
28. Widowati W, Mozef T, Risdian C, Ratnawati H, Tjahjani S, Sandra F. The Comparison of Antioxidative and Proliferation Inhibitor Properties of Piper betle L., Catharanthus roseus [L] G.Don, Dendrophtoe petandra L., Curcuma mangga Val. Extracts on T47D Cancer Cell Line. Int Res J Biochem Bioinform. 2011; 1: 22-8.
29. Arung ET, Kusuma IW, Purwatiningsih S, Roh SS, Yang CH, Jeon S, *et al.* Antioxidant Activity and Cytotoxicity of the Traditional Indonesian Medicine Tahongai (Kleinhovia hospita L.) Extract. J Acupunct Meridian Stud. 2009; 2: 306-8.
30. Wicaksono BD, Arung ET, Sandra F. Aktivitas Antikanker dari Kayu Secang. CDK. 2008; 35: 133-7.
31. Widowati W, Maesaroh, Fauziah N, Erawijantari PP, Sandra F. Free Radical Scavenging and Alpha/Beta-glucosidases Inhibitory Activities of Rambutan (Nephelium lappaceum L.) Peel Extract. Indones Biomed J. 2015; 7: 157-62.
32. Wicaksono BD, Tangkearung E, Sandra F. Brucea javanica leaf extract induced apoptosis in human oral squamous cell carcinoma (HSC2) cells by attenuation of mitochondrial membrane permeability. Indones Biomed J. 2015; 7: 107-10.
33. Rizal MI, Sandra F. Brucea javanica leaf extract activates caspase-9 and caspase-3 of mitochondrial apoptotic pathway in human oral squamous cell carcinoma. Indones Biomed J. 2016; 8: 43-8.
34. Arung ET, Wicaksono BD, Handoko A, Kusuma IW, Yulia D, Sandra F. Anti-cancer properties of diethylether extract of wood from sukun (Artocarpus altilis) in human breast cancer (T47D) cells. Trop J Pharm Res. 2009; 8: 317-24.
35. Wicaksono BD, Handoko A, Arung ET, Kusuma IW, Yulia D, Pancaputra AN, *et al.* Antiproliferative effect of methanol extract of Piper crocatum Ruiz & Pav leaves on human breast (T47D) cells in-vitro. Trop J Pharm Res. 2009; 8: 345-52.
36. Arung ET, Wicaksono BD, Sandra F. Prenylated Flavonoid sebagai Senyawa Anti Kanker yang Berpotensi. CDK. 2009; 36: 20-2.
37. Tendean M, Oktaviono YH, Sandra F. Cardiomyocyte Reprogramming: A Potential Strategy for Cardiac Regeneration. Mol Cell Biomed Sci. 2017; 1: 1-5.
38. Wijaya L, Agustina D, Lizandi AO, Kartawinata MM, Sandra F. Reversing breast cancer stem cell into breast somatic stem cell. Curr Pharm Biotechnol. 2011; 12: 189-95.
39. Puspitasari RL, Boediono A, Sandra F. Conditioned Medium dari Kultur Primer Sel Syaraf Mus musculus. Prosiding Seminar Biologi. 2013; 10: 1-6.
40. Sandra F, Sudiono J, Sidharta EA, Sunata EP, Sungkono DJ, Dirgantara Y, Chouw A. Conditioned Media of Human Umbilical Cord Blood Mesenchymal Stem Cell-derived Secretome Induced Apoptosis and Inhibited Growth of HeLa Cells. Indones Biomed J. 2014; 6: 57-62.