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Anticancer Activity of Trisindolina 1 by Inducing Apoptotic Pathway Through Phosphorylated p53 Protein Expression

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ABSTRACT

Cancer is an abnormal proliferation that may arise from normal cells due to carcinogenic induction which causes oxidative stress and damaging DNA. Damaged DNA can be repaired in the G1 phase of the cell cycle, or can be eliminated by directing cell to the apoptotic death pathway. Both mechanisms are controlled by tumour suppressor protein p53. Several natural substances are known to have anticancer potential by inducing p53 expression, one of which is Trisindolina 1 that shows cytotoxic effect in several cancer cell lines. In this paper, we review progress on possible anticancer mechanism of Trisindolina 1, mainly by inducing expression of phosphorylated p53 in apoptotic pathway.

Key words: *Apoptosis, Cancer, Phosphorylated p53, Trisindolina 1.*

Introduction

Cancer is a major public health problem and one of the leading cause of mortality with estimated 18 million new cases and 9.5 million deaths worldwide in 2018 (Bray *et al.,* 2018; Habli *et al.,* 2017). The main characteristic of cancer are uncontrolled cell growth and acquisition of metastatic properties, however inactivity of apoptosis mechanism can also be found. In most cases, cancer might originated from activation of oncogenes and/or deactivation of tumor suppressor genes induced by several stressor (Sarkar *et al.,* 2013; Pitolli *et al.,* 2019) which result is an impaired cells, genes and signaling pathways that lead to irregular cell proliferation mechanisms and resistance to apoptosis (Hanahan and Weinberg, 2011).

Apoptosis is programmed death in cells both in physiological and pathological conditions that are regulated by several kinds of proteins, one of which is the p53 protein encoded by the TP53 gene. This protein induces apoptosis when there are cells that have the potential to cause tumors or cancer (have genetic errors or changes) (Wong, 2011; Vieler and Sanyal, 2018). Thus, apoptosis is one of the vital pathways through which chemo-preventive agents inhibit the growth of cancer cells. The ability of p53 to induce apoptosis can be used as a target for the therapy and development of cancer drugs and it is important to investigate whether the drugs are associated with the induction of apoptosis and alterations of apoptosis related gene expression (Wong, 2011; Rahman *et al.,* 2000).

Currently natural bioactive substances are getting a lot of investigation as main interest in producing

novel anticancer chemotherapeutic agents (Al-Wabli *et al.,* 2020). One of the natural substance in question are from marine sources. Novel molecules based on natural heterocyclic scaffold, which are indole and its derivatives, have been shown to exhibit significant antiproliferative and anticancer activity. As the reservoir of living organisms is limited, it is necessary to synthesize biologically active natural molecules and their derivatives to meet the demand in those medicinal agents (Eldenha *et al.,* 2020). Trisindolina is a secondary metabolite in the form of indole trimer which was first isolated from *Vibrio* sp. on the sponge *Hyrtios altum* in Okinawa, Japan and has been successfully synthesized according to its natural form (Kobayashi *et al.,* 1994; Mustikasari and Santoso, 2012). The high potential cytotoxicity of Trisindolina compounds has led to many studies on the cytotoxicity levels of Trisindolina derivatives by adding functional groups such as halogens (Yoo *et al.,* 2008; Fortes, *et al.,* 2016). In this paper, we review progress on possible anticancer mechanism of newly synthetized halogenated Trisindolina in several cancer cell lines, mainly by inducing expression of phosphorylated p53 in apoptotic pathway.

p53 Protein and Cancer

The transcription factor p53 is a tumour suppressor that is an essential regulator of cellular stress responses. In response to a variety of cellular stresses including DNA damage, the p53–Mdm^2 interaction is disrupted and p53 is rapidly stabilized (Carvajal and Manfredi, 2013; Levine, 2019). The accumulated p53 protein is subject to extensive post-translational modifications including phosphorylation, acetylation, etc. Stabilized p53 is localized to the nucleus in which it can activate or repress the transcription of many genes involved in regulating the main cellular responses to stress, such as cell cycle arrest, DNA repair, senescence and apoptosis, among others (Pitolli *et al.,* 2019; Vieler and Sanyal, 2018). Because it controls many cell-fate-deciding genes, p53 has a prominent role for the diagnosis and the treatment in cancer. The cells with DNA damage, whose normal fate is to die, can survive due to inactivation of p53 and still divide irregularly which leads to cancer. The major cause on how p53 can be inactivated is DNA binding domain (DBD) mutations. This makes the protein fail to bind to the target DNA (Pitolli *et al.,* 2019; Vieler and Sanyal, 2018).

p53 Protein Structure

p53 is a tetrameric protein composed of 393 amino acids (numbered from amino terminus to carboxy terminus) which commonly tetramerizes with four identical subunits and acts as a transcription factor. This protein itself has a modular structure comprising of six domains (Vieler and Sanyal, 2018).

At the amino terminus are the two transactivation domains I and II (TAD I, and TAD II), spanning residues 1–67. Amino acid 22 and 23 in TAD I are essential for binding the MDM2 ubiquitin ligase. The two N-terminal transactivation domains can independently activate transcription of the target genes. These TADs are not equivalent and are required to induce different promotors. The TAD domain is followed by the proline-rich region (PRD) with residues 68–98 that links TAD to DBD and is required for apoptosis and growth suppression triggered by p53. The DNA-binding domain (DBD, also called the core domain) with residues 94–292 are containing several arginine amino acids and one zinc atom which interact with DNA molecule in sequence-specific manner and where mutations in the TP53 gene are mainly found in cancer cells (Vieler and Sanyal, 2018; Levine, 2019). The hinge domain (HD), with residues 293–325, next is the oligomerization domain (OD) with residues 326–353. The OD contains the nuclear export signal (NES), which is masked by tetramerization of p53 to prevent from exporting to the cytoplasm. After synthesis, p53 monomer assembles into a dimer and OD assemble the protein into a dimer of a dimer (producing tetramer) by forming salt bridges and stabilize the tetramer. This domain helps to deform the bound DNA and thereby facilitate DBD for stable binding of DNA. Last domain is the carboxy-terminal regulatory domain (CTD) spanning residues 353–393. This domain composed of several lysine residue that involved in the down-regulation of the central DNA binding domain by undergo posttranslational modification (methylation to inactivate transcription activity or acetylation to activate transcription), therefore controls the structure and function of the entire Protein (Vieler and Sanyal, 2018; Levine, 2019; Bai and Whu, 2006).

p53 Posttranslational Modification

In response to DNA damage, p53 undergoes a series of post-translational modifications thought to regu-

late its stability and biological functions. These modifications can be in the form of phosphorylation, acetylation, methylation, ubicuitination, and many others. The function of this modification is to stabilize the p53 protein so that it can be more active in playing its role as a transcription factor (Vieler and Sanyal, 2018; Carvajal and Manfredi, 2013; Jenkins, *et al.,* 2012).

Phosphorylation of proteins is a common post-translational modification. In general, phosphorylation occurs by adding a phosphate group (PO_4) to the polar group R in amino acids with the help of the kinase enzyme. This modification causes the protein to become hydrophilic and polar so that it is able to interact with other molecules or proteins (PPIs). In the p53 protein, phosphorylation can be found at more than 20 locations along the TAD domain at the N end, the linker domain and the REG domain at the C end (Jenkins *et al.,* 2012; MacLaine and Hupp, 2011; Ardito *et al.,* 2017). Ultraviolet (UV) radiation has been reported to induce Ser33 and Ser46 phosphorylation on the amino-terminus of p53 by the p38 kinase. Ser46 phosphorylation is required for activation of the pro-apoptotic factor p53AIP1 in response to high level of DNA damage and was shown that p53AIP1 localizes to the mitochondria and facilitates the release of cytochrome c during apoptosis (Carvajal and Manfredi, 2013; Oda *et al.,* 2000).

The nuclear serine/threonine kinase, homeodomain-interacting protein kinase 2 (HIPK2), was reported inducing phosphorylation of p53 on Ser46 in response to severe DNA damage where reduced Mdm2 levels result in the accumulation of HIPK2 and triggering UV-induced transcriptional upregulation of pro-apoptotic genes and apoptosis (Carvajal and Manfredi, 2013; D'Orazi *et al.,* 2002; Rinaldo *et al.,* 2007).

DYRK2 is another kinase reported to phosphorylate p53 on Ser46 that leads to p53-dependent apoptosis (Carvajal and Manfredi, 2013). Upon DNA damage, ataxia telangiectasia mutated (ATM) phosphorylated and stabilized DYRK2 in the nucleus, then, DYRK2 phosphorylated p53 at Ser46 to induce apoptotic mechanism (Yogasawa and Yoshida, 2018).

Ser15 was reported to be a phosphorylation target of the protein kinases DNA-PK and ATM. It has been suggested that phosphorylation of p53 on Ser15 by ATM promotes phosphorylation at Ser6, Ser9, Thre18, Ser20 and Ser46 which are required for p53 stabilization and efficient induction of apoptosis (Carvajal and Manfredi, 2013; MacLaine and Hupp, 2011).

Apoptosis

Apoptosis is programmed death in cells, both in physiological and pathological conditions, which are regulated by several kinds of proteins. The p53 protein is one of the tumor suppressor proteins that induces apoptosis in presence of genetically errors or impaired cells (Wong, 2011; Vieler and Sanyal, 2018). Apoptosis induced by p53 is an intrinsic pathway which activated by intracellular events that inducing the release of pro-apoptotic factors from the mitochondria such as cytochrome c accompanied by the activation of caspase 9, -8 and -3 (Elmore, 2007; Salucci *et al.,* 2018).

In response to low levels of DNA damage, a small amount of p53 protein will be released to promote cell cycle arrest, but when a high level of DNA damage present, a large amount of p53 protein will be released to nucleus and sustained which leads to apoptosis (Carvajal and Manfredi, 2013). The induction of p53 in apoptosis is by inhibiting Bcl-2 expression and increasing Bax expression. Bcl-2 family is closely involved in the regulation of cytochrome c release into the cytosol. Bcl-2 preserve the integrity of mitochondria and block the release of cytochrome c (Salucci *et al.,* 2018). In response to apoptotic signals, Bax which located in the cytosol translocated to the outer membrane of mitochondria and heterodimerizing with Bcl-2. This makes membrane more permeable and began to releasing cytochrome c into the cytosol (Salucci *et al.,* 2018). Cytochrome c will bind to Apaf-1 protein and triggering activation of procaspase-9 into caspase 9. This apoptosome will trigger the caspases activation. Caspases are a family of cysteases, which cleave protein substrates after their Aspartic acid residues and are involved in regulating the activation of apoptotic signal transmission. This family, once activated, can often activate other procaspases, allowing initiation of a protease cascade. The apoptosome induce activation of caspase 3, which in turn causing chromatin condensation, induces cytoskeletal reorganization and disintegrating cells into apoptotic bodies (Wong, 2011; Elmore, 2007).

It is known that one of the cancer hallmark is avoiding apoptosis and might happen by upregulation of anti-apoptotic proteins and/or by downregulation or dysfunction of proapoptotic mol-

ecules (Fulda, 2010; Hanahan and Weinberg, 2011). The mitochondrial pathway Bcl-2 family consist both of anti- and pro-apoptotic proteins and its right ratio are dictate the regulation in apoptosis. This correspondence to a finding that an increase in the ratio of anti- to proapoptotic Bcl-2 proteins has been detected in various cancers and are correlated to tumor cell survival and apoptosis resistance (Adams and Cory, 2007; Chen and Pervaiz, 2009).

Indole-Based Cancer Drugs

As discussed above, the involvement of apoptosis and p53 in the pathophysiology of cancer are benefitting as a target for the therapy and development of cancer drugs (Wong, 2011). Currently natural bioactive substances are getting a lot investigation as main interest in producing novel anticancer chemotherapeutic agents (Al-Wabli *et al.,* 2020). One of the natural substance in question are from marine sources that are rich in indole scaffold and has undergone extensive development (Eldenha *et al.,* 2020; Yoo, *et al.,* 2008; Mustikasari and Santoso, 2016; Netz and Opatz, 2015; Yuan *et al.,* 2019). Diverse substituted indole and bis-indole derivatives extracted from marine source have been shown to exhibit significant antiproliferative and anticancer activity (Eldenha *et al.,* 2020; Salucci *et al.,* 2018).

Trisindolina is a secondary metabolite in the form of indole trimer which was first isolated from *Vibrio* sp. on the sponge *Hyrtios altum* in Okinawa, Japan and has been successfully synthesized according to its natural form (Kobayashi *et al.,* 1994; Yoo *et al.,* 2008; Mustikasari and Santoso, 2012). The high potential cytotoxicity of Trisindolina compounds has led to many studies on the cytotoxicity levels of Trisindolina derivatives by substitution such as halogens and some other functional group, etc (Yoo *et al.,* 2008; Fortes *et al.,* 2016; Mursyidah and Samtoso, 2010; Varun *et al.,* 2019; Susanti, 2020). Recent study (Susanti, 2020) reported that new derivative, Trisindolina 1, were potential towards HepG2 cell line by inducing growth arrest and expressing p53 that leads to activation of apoptosis pathway. This level of toxicity might originated from indole metabolism. The oxidation of indole compounds will form indoxyl and isatin which further might condensed to form toxic indirubin compounds (Yoo, *et al.,* 2008; Medvedev *et al.,* 2007; Bla•evic *et al.,* 2015). Indirubin and its derivatives were found to have potential uses as anticancer drugs as they have been reported to inhibit the activities of cyclin-dependent kinases, key controllers of cell cycle progression in eukaryotic cells (Yoo *et al.,* 2008). Other studies reported in (Fogaça *et al.,* 2017) suggest that indirubin also inhibit glycogen synthase kinase 3b (GSK3b) in tumour cells, resulting in an impairment of cell cycle progression, inducing apoptosis by disabling activation of Stat3 (a transcription factor that controls cell proliferation and survival), and also interfering with the activity of protein kinase B (Akt), extracellular signal-regulated kinases (Erk), Notch1 and cytokines resulting in antiproliferative effect. In the same direction, isatin inhibits cell proliferation and induces apoptosis in mouse and human neuroblastoma cells by altering Erk signalling, and able to down-regulate Bcl-2 thus disrupting mitochondrial membrane and releasing cytochrome c that leads to activation of pro-apoptotic caspase cascade, resulting in apoptosis (Fogaça *et al.,* 2017; Ma *et al.,* 2014). Indole-based compound with halogen substitution demonstrated a higher antitumor activity compared to the non-substituted compound (Fortes *et al.,* 2016; Bla•evic *et al.,* 2015). It is reported that the C-5 position of isatin were suitable for substitution and halogen atoms are are more beneficial to the activity. Isatin dimer, indirubin, with substituent halogen atom nitro and fluoro could enhance the anticancer activity against several cancer cell lines (Bla•eviæ *et al.,* 2015; Zhang *et al.,* 2020).

To summarize the possible pathway of Trisindolina 1 anticancer activity as indole/isatin/indirubin derivative, we suggest several schematic. (i) Trisindolina inducing stress in cell, increasing p53 and inhibit cyclin-dependent kinases that leads to cell cycle arrest and downregulate of Bcl-2, disrupting mitochondria membrane integrity. Cytochrome c then released from mitochondria then inducing activation of pro-apoptotic protein thus triggering apoptosis; (ii) Trisindolina damaging DNA causing accumulation of HIPK2 in nucleus which disrupting Mdm2-p53 complex and promote phosphorylation of p53 and resulting in transcription of pro-apoptotic genes, leads to apoptosis; (iii)Trisindolina damaging DNA and inducing phosphorylation of ATM which stabilize DYRK2, promoting phosphorylation of p53 that leads to triggering apoptosis pathway; (iv) Trisindolina disrupting Erk signaling, leads to cell growth and proliferation arrest.

Conclusion

Apoptosis, which regulated by p53 protein, is one of the vital pathways to inhibit cancer cells through

chemo-preventive agents. Indole group are known cytotoxic toward several cancer lines through apoptosis pathway and its derivative are being developed. One of these agents is trisindolina, an indole-based compound from marine source, and its derivative Trisindolina 1, were reported potential as anticancer agent toward several cancer cell lines. Recent studies on Trisndolina 1 are determine which apoptosis pathway induces cell death in cancer cells. As indole/isatin/indirubin derivative and several studies on the trisindolina, this paper review propose several possible pathway of Trisindolina 1 anticancer activity which involve upregulation of p53 protein and its activation via phosphorylation that further trigger upregulation and activation of pro-apoptotic proteins, which leads to apoptotic death pathway.

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